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ERRATA FROM PREVIOUS VOLUMES

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|--------------------|--|--|
| p. 97, lines 37–38 | <i>for:</i> unpigmented lower two cells. | <i>read:</i> pigmented basal cell. |
| p. 411, line 15 | <i>for:</i> Korea Forest Institute | <i>read:</i> Korea Forest Research Institute |

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FROM THE EDITORS

MYCOTAXON, DELAYS, AND 2012 — In 1973 Dick Korf and Gregoire Hennebert noted that taxonomic papers were unnecessarily delayed by traditional blind peer review and press processing. They developed MYCOTAXON to eliminate these frustrating bottlenecks by having authors select their own expert reviewers (thus directing the review pace) and prepare print ready copy. This innovative procedure was enthusiastically received. Papers were published quickly, usually within three months after submission, and MYCOTAXON established an enviable reputation for reliability and efficiency. Complaints, if any, focused on the ‘unprofessional’ appearance of the journal. Begun in the days of typewritten copy, the journal permitted each author to select their own style and formatting, resulting in a somewhat ‘haphazard’ appearance for each volume.

Nine years ago, our Editorial Advisory Committee suggested that ‘MYCOTAXON style’ move from oxymoron to reality. With authors more or less computer literate, the new *Editor-in-Chief* could require electronic submission and expand the ‘Instructions to Authors’ to set forth the new style and formatting requirements. Happily, by early 2005, all submissions arrived on the editorial desk via Email, except for an occasional illustration-heavy CD sent by regular post.

Adding our *Nomenclature Editor* in 2005 ensured thoroughly vetted nomenclature; with two editors revising each manuscript, fewer errors were published and most papers were published 3 to 6 months after final submission. Our impact factor rose steadily through 2011.

We still strive to process manuscripts and publish as speedily as possible. Unfortunately our speed and efficiency rest on a manageable number of manuscripts (~250 annually) & emails— and Shaun’s and my health. 2012 has brought a ‘perfect storm’ for delays – manuscripts pouring in at an accelerated rate and unexpected medical ‘issues’ for both Editors. The early April publication of MYCOTAXON 119 was followed by eye surgery and major orthopedic surgery for Shaun, worrying authors accustomed to nomenclatural reviews returned within 1–2 weeks (needlessly so, however, as all manuscripts are still processed in the order received). Shaun fortunately is now back at the nomenclatural desk and hard at work on the 70+ manuscripts that accumulated during his recuperations.

Understandably, my treatment of the cancer diagnosed in July HAS delayed this volume by three months, as I can no longer put in the 70–80 hours per week on Email and revising, reformatting, and processing manuscripts that I have since 2004. With my final reviews for MYCOTAXON 121 only now beginning, the July–September volume will also be late. Ever optimistic, however, I still hope that Mycotaxon 122 will be delayed only one month, (not three!). [I dream!]

We take this opportunity to thank our authors for their patience and understanding. We feel that MYCOTAXON remains the most comprehensive journal for fungal taxonomy and nomenclature. It should remain so for years to come and should arrive once more in a predictably timely fashion after resuming its normal schedule in 2013.

Warm regards,
 LORELEI NORVELL (*Editor-in-Chief*) with
 SHAUN PENNYCOOK (*Nomenclature Editor*)
 22 September 2012

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April–June 2012

***Glomus truffemii* (Glomeromycetes), a new sporocarpic species from Brazilian sand dunes**BRUNO TOMIO GOTO^{1*}, JOMAR GOMES JARDIM¹, GLADSTONE ALVES DA SILVA²,
EDUARDO FURRAZOLA³, YAMIR TORRES-ARIAS³ & FRITZ OEHL⁴¹*Departamento de Botânica, Ecologia e Zoologia, CB, Universidade Federal do Rio Grande do Norte, Campus Universitário, 59072-970, Natal, RN, Brazil*²*Departamento de Micologia, CCB, Universidade Federal de Pernambuco, Av. Prof. Nelson Chaves, S/N, CEP 50670-420, Cidade Universitária, Recife, PE, Brazil*³*Instituto de Ecología e Sistemática, IES-CITMA, A.P. 11900, La Habana, Cuba*⁴*Federal Research Institute Agroscope Reckenholz-Tänikon ART, Organic Farming Systems, Reckenholzstrasse 191, CH-8046 Zürich, Switzerland**CORRESPONDENCE TO: brunogoto@hotmail.com

ABSTRACT — *Glomus truffemii*, which forms large aggregates ($\leq 850 \times 1200 \mu\text{m}$) in the rhizosphere of a herbaceous plant community in NE Brazilian sand dunes, is described as new. Its subglobose glomerospores measuring $72\text{--}92 \times 79\text{--}105 \mu\text{m}$ have two spore wall layers: an evanescent hyaline $0.3\text{--}0.8 \mu\text{m}$ thick outer layer and a laminate orange brown to dark red brown $7.4\text{--}15.5 \mu\text{m}$ thick inner layer. The pigmentation of the subtending hypha is similar but often much lighter than that of the laminate spore wall layer. The spore size and color, spore wall structure, and features of the spore base and subtending hyphae separate this species from similar yellow-brown to brown spored species like *G. badium*, *G. glomerulatum*, and *G. brohultii*.

KEY WORDS — morphology, *Glomeromycota*, *Glomerales*, restinga

Introduction

The Tulasne brothers described the genus *Glomus* in 1844 for *G. macrocarpum* (the type species according to Clements & Shear 1931). Since then many other fungi with glomoid spores like those of *G. macrocarpum* have been named, so that now *Glomus* comprises >60 species. For many years *Glomus* was considered monophyletic based solely on morphological evidence. Morton & Redecker (2001), based on morphological and molecular data, erected the genera *Paraglomus* (*Paraglomeraceae*) and *Archaeospora* (*Archaeosporaceae*), whose members also formed glomoid spores. Schwarzott et al. (2001) inferred from phylogenetic SSU rDNA molecular analyses that *Glomus* is non-

monophyletic. Schüßler et al. (2001) subsequently erected a new phylum, *Glomeromycota*, in which *Glomus* was represented by three phylogenetically different groups: A, B, and C. Oehl & Sieverding (2004) transferred glomoid species with two spore walls (with the inner wall serving as a germinal wall) to their new genus *Pacispora*. Thereafter Walker & Schüßler (2004) transferred *Glomus spurcum* C.M. Pfeiff. et al. to the newly established genus *Diversispora* and order *Diversisporales*.

To date, glomoid glomerospores can be found in species representing *Archaeosporales*, *Diversisporales*, *Glomerales*, and *Paraglomerales*, making their identification difficult in the absence of molecular data. Morphological identification has been facilitated recently in a thorough review of glomoid spore-forming species (Oehl et al. 2011), which introduced helpful suggestions based on molecular data associated with morphological differences among the major phylogenetic clades in the *Glomeromycota*. Our description of *G. trufemii* is based exclusively on the new key morphological criteria, and the species fits the Oehl's et al. (2011) revised concept of *Glomus*.

Material & methods

Study area and sites

Samples were collected in the Parque Estadual das Dunas de Natal “Jornalista Luiz Maria Alves,” the first Conservation Unit created in the Municipality of Natal, Rio Grande do Norte State and currently one of the largest urban conservation areas with dune vegetation in Brazil. The site is located at 5°46'S 35°12'W with soils analyzed as pH (H₂O) = 5.5–5.75, P = 2–4 mg kg⁻¹, 2,86 g dm³ of organic matter. The climate is tropical rainy (type Am of Köppen) with a short four-month dry period, with a 25.5 °C mean annual temperature and 1191 mm mean annual precipitation. In the sand dune ecosystem, the typical ‘restinga’ vegetation varies from herbaceous to shrubs and trees (Oliveira-Filho 1993, Oliveira-Filho & Carvalho 1993). The park is composed of mainly represented by tree species of the *Anacardiaceae*, *Bignoniaceae*, *Fabaceae*, *Myrtaceae*, and *Rubiaceae* (*Anacardium occidentale*, *Caesalpinia echinata*, *Campomanesia dichotoma*, *Chamaecrista ensiformis*, *Eugenia ligustrina*, *Guettarda platypoda*, *Myrcia guianensis*, *Myrciaria tenella*, *Tabebuia roseoalba*, *Tocoyena sellowiana*) and herbaceous species of *Poaceae*, *Cyperaceae*, *Asteraceae*, *Araceae*, and *Rubiaceae*.

Morphological analyses

Glomerospores and sporocarps were separated from the soil samples by sucrose gradient in plate dishes (Błaszczkowski et al. 2006). Sporocarps and glomerospores were placed in dishes with water and separated under a dissecting microscope. Sporocarps were fragmented to study spore organization. Glomerospores were mounted on microscope slides either in water (to check unmodified characteristics of spore wall components and spore colour; Spain 1990) or permanently in PVLG (Omar et al. 1979) or PVLG with Melzer's reagent (Koske & Tessier 1983).

Terminology follows Oehl et al. (2011) and Furrázola et al. (2011). The spore denomination of Goto & Maia (2006) was used. Zeiss Axioskop compound microscopes with or without

Nomarski differential interference contrast (DIC) were used for detailed spore observations; digital images were taken with either an AxioCam camera and AxioVision (v. 3.1 software at 1300 × 1030 dpi) or Canon digital cameras.

Arbuscular mycorrhizal cultures

Bait cultures for the soils from sand dunes (Parque das Dunas de Natal) with spores and sporocarps were established in the greenhouse of the Universidade Federal do Rio Grande do Norte with *Zea mays* L. as host plant, using sterilized soil and 0.5 L pots.

Pure cultures of the new fungus were attempted by inoculating *Zea mays* seedlings germinated in autoclaved quartz sand with *G. trufemii* sporocarps isolated from sand dunes. After filling 0.5 L pots with sterilized sand dune soil, we formed a small hole in the center using a glass shaker and discharged spores and sporocarps into the hole using Pasteur pipettes. Then a *Z. mays* seedling was placed into the hole, covered with sand, and grown for one cycle of 3–4 months. The fungus did not produce spores in either bait cultures grown even for 9–12 months or pure cultures.

Taxonomy

Glomus trufemii B.T. Goto, G.A. Silva & Oehl, sp. nov.

FIGS. 1–9

MYCOBANK MB 561567

Differs from *G. brohultii* by more regularly shaped subtending hyphae that are pigmented further below the spore bases and darker colored orange to red brown spores with slightly thicker walls.

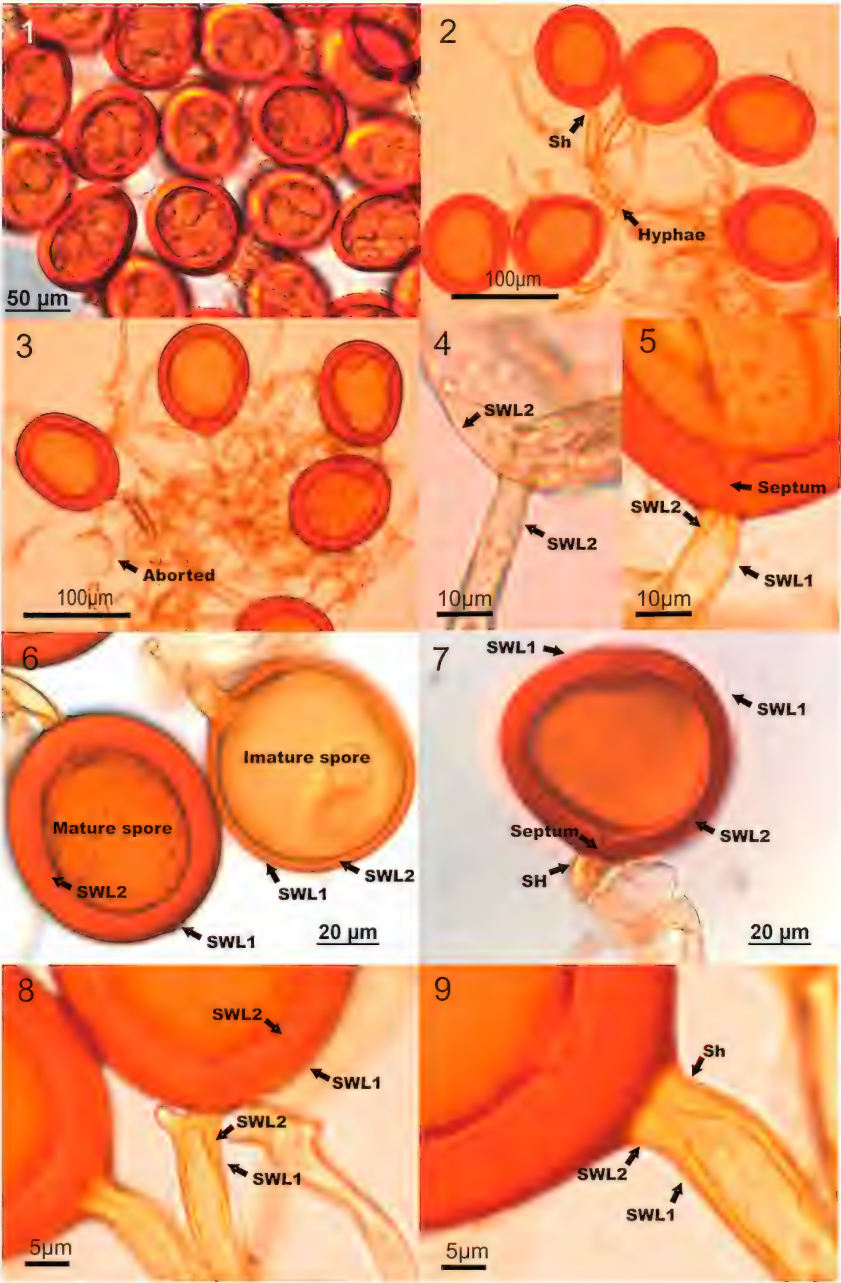
TYPE: Brazil, Rio Grande do Norte, near Natal, Parque das Dunas de “Natal”, sand dunes with herbaceous plant community on a convex hillside, 15.Feb.2010, B.T. Goto, in permanent slide (PVLG) (**Holotype**: UFRN1482; **Isotype**: UFRN1483, ZT Myc 15118).

ETYMOLOGY: *trufemii* = in honor of Sandra Farto Botelho Trufem, taxonomist, who long taught many young students and researchers in arbuscular mycorrhizal fungal taxonomy.

SPOROCARPS formed in aggregates (500–850 × 780–1200 µm), orange brown to dark red brown adherent to living or dead roots with hundreds to thousands of glomerospores; rarely singly in soils. Melzer’s reaction not observed. Peridium absent. Sporocarpic hyphae hyaline (3.8–)7.6–10.4(–12.8) µm without a septum and with interwoven arrangements.

GLOMEROSPORES formed terminally on hyphae, subglobose to elliptical (72–92 × 79–105 µm) or rarely globose (72–96 µm diam.), orange brown when young to dark red brown at maturity, perhaps darkening slightly to dark brown when ageing in soil.

SPORE WALL 7.4–15.5 µm thick overall, consisting of two layers (SWL1, SWL2); SWL1 thin (0.3–0.8 µm), hyaline and difficult to observe in mature spore, but generally visible in the subtending hyphal wall; SWL2 thick (7.4–15.5 µm), pigmented orange brown to dark red brown, laminated, smooth. Spore wall layers continuous with subtending hyphal wall layers. The pigmentation of SWL2 continues into the subtending hyphal wall, but this layer is often lighter



in color in the subtending hypha. SWL1 and SWL2 do not stain when exposed to Melzer's reagent.

SUBTENDING HYPHA (SH) generally present, single, straight or constricted, cylindrical to sharply curved, light yellow to dark yellow; 5.1–12.7 μm diam. (mean-8.8 μm) at the spore base, the hyphal wall 2.6–5.1 μm thick (mean 4.2 μm) near the spore base and tapering to approx. 1 μm at 15 μm from the base; occlusion formed by introverted SWL2 thickening and often through an additional bridging septum arising from SWL2, but sometimes the pore appearing partly open.

ABORTED GLOMEROSPORES frequently observed and forming terminally on hyphae, generally collapsed or subglobose to elliptical (35–56 $\times$ 40–46 μm), light yellow to dark yellow, possibly darkening slightly to light brown when aging in soils. Spore wall layer (SWL2) (<3.8 μm) not staining in Melzer's reagent.

GERMINATION structures unknown.

GLOMEROSPORE DEVELOPMENT was deduced from positively identified spores in aggregates found in different developmental stages. The hyaline hyphal wall layer differentiates into a hyaline, evanescent spore wall layer (SWL1) and then a laminate layer (SWL2) that becomes more pigmented with increasing numbers of developing sublaminae. After the glomerospores mature, their pore is closed by introverted thickening of SWL2 and an additional bridging septum arising from the laminate wall layer.

ARBUSCULAR MYCORRHIZA FORMATION remains unknown.

DISTRIBUTION — So far, the new fungus was detected only in Brazil. Known in soil from sand dunes mainly harbouring herbaceous species of *Araceae*, *Asteraceae*, *Cyperaceae*, *Poaceae* and *Rubiaceae* (e.g.: *Acroceras zizanioides* (Kunth) Dandy, *Anthurium affine* Schott, *Aspilia procumbens* Baker, *Centratherum punctatum* Cass., *Cyperus maritimus* Poir., *C. meyenianus* Kunth, *Mitracarpus eichleri* K.Schum., *Raddia brasiliensis* Bertol., *Richardia grandiflora* (Cham. & Schltdl.) Steud.) growing in native, ecologically unstable restinga ecosystems in Parque das Dunas, Natal (Brazil).

Discussion

Glomus species are very common in sand dunes (Błaszowski 2010; Błaszowski et al. 2009a,b, 2010a,b) and several new species have been described recently (*G. africanum* Błasz. & Kovács, *G. achrum* Błasz. et al.,

FIGS. 1–9. *Glomus truffemii*. 1. Sporocarps in PVLG; note the numerous glomerospores in this part of sporocarp. 2–3. Fragment of sporocarp with healthy glomerospores and aborted spores. 4. Aborted spores with only one spore wall layer (SWL2). 5. Septum formed by SWL2. 6. Spores in different developmental stages. 7. Spore wall layers in mature spore (SWL1&2). 8–9. Spore wall layers (SWL1&2) continuous with subtending hyphal wall (SW) that form distinct pigmentation.

G. bistratum Błaszcz. et al., *G. indicum* Błaszcz. et al., *G. iranicum* Błaszcz. et al., *G. majewskii* Błaszcz., *G. perpusillum* Błaszcz. & Kovács). *Glomus trufemii* is readily distinguished from previously described sporocarpic *Glomus* species by glomerospore size and colour, spore wall structure, (including that at the spore base), and characters of the subtending hypha. Most of these species form colourless or pale-coloured spores singly or in loose aggregates (Błaszczowski et al. 2009a,b; 2010a,b) and thus are easy to distinguish from the sporocarpic dark-spored *G. trufemii*. Sporocarps of *G. trufemii* slightly resemble spore aggregates of *G. aggregatum* N.C. Schenck & G.S. Sm. and *G. fasciculatum* (Thaxt.) Gerd. & Trappe (Schenck & Smith 1982, Gerdemann & Trappe 1974). However, the *G. fasciculatum* SWL2 stains in Melzer's reagent (vs. no reaction in *G. trufemii*). Spores of *G. aggregatum* generally are irregular in shape (vs. globose to subglobose) and have much thinner spore and subtending hyphal walls. The spore wall of *G. trufemii* is one of the thickest among the known glomeromycotan species with glomoid spores.

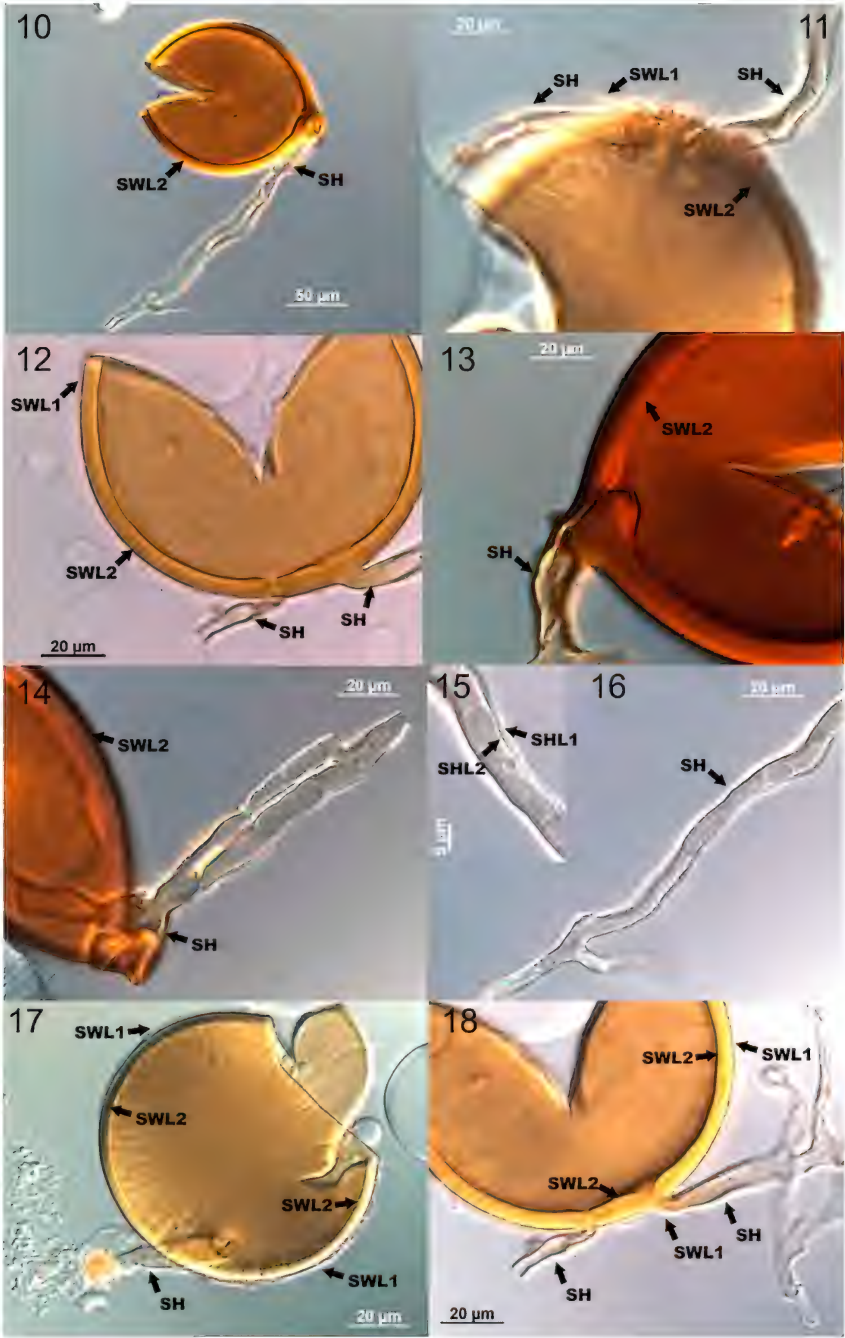
Several *Glomus* spp. form sporocarps without a peridium with similarly sized and coloured spores similarly distributed in a sporocarp: *G. ambisporum* G.S. Sm. & N.C. Schenck, *G. atrouva* McGee & Pattinson, *G. badium* Oehl et al., *G. boreale* (Thaxt.) Trappe & Gerd., *G. botryoides* F.M. Rothwell & Victor, *G. brohultii* Sieverd. & R.A. Herrera, *G. flavisporum* (M. Lange & E.M. Lund) Trappe & Gerd., *G. glomerulatum* Sieverd., *G. heterosporum* G.S. Sm. & N.C. Schenck, *G. invermaium* I.R. Hall, and *G. macrocarpum* Tul. & C. Tul. (Oehl et al. 2011). However, of these only *G. badium*, *G. brohultii*, and *G. glomerulatum* also form spores with subtending hyphae generally clearly lighter-coloured than the spore wall (Sieverd. 1987, Herrera-Peraza et al. 2003, Oehl et al. 2005, Oehl et al. 2011). In the other species, the wall color of both spore and subtending hyphae is similar or identical (Oehl et al. 2011).

Glomus glomerulatum produces intercalary yellow brown glomerospores having 2–4 subtending hyphae (vs only terminal spores with one subtending hypha in *G. trufemii*). Moreover, *G. trufemii* spores are darker (orange-brown to dark reddish brown) and have a significantly thicker wall.

Glomus badium forms irregularly sized 3-layered spores around an intrasporocarpic gleba, while *G. trufemii* generally produces regularly sized subglobose 2-layered spores in sporocarps lacking glebas.

Glomus trufemii and *G. brohultii* spores have a similar size and wall structure. Also, although *G. brohultii* was not originally described as forming sporocarps

FIGS. 10–18. *Glomus brohultii*. Bi-layered spores (swL1&2). 10. Glomerospores in PVLG; note the subtending hypha (SH) which is pigmented up to ca. 50 µm below the spore base before becoming subhyaline to hyaline. 11–12. Spores frequently have two subtending hyphae. 13. Mature spore with swL2 thickened at the spore base. 14. Irregular subtending hypha becoming hyaline in a rather short distance from the spore base (20–30 µm). 16. Hyaline subtending hypha with two layers (shL1&2). 17–18. Crushed spores with two spore wall layers (swL1&2).



or aggregates, sporocarps have been observed recently (Oehl, pers. obs.) during AMF diversity studies in cultivated white and yellow yam sites and adjacent natural savannas in West Africa (Tchabi et al. 2009), ruling out sporocarp or aggregate formation as a diagnostic character separating *G. trufemii* and *G. brohultii*. However, *G. brohultii* spores are paler and lack orange or red ones— yellow-brown to brown, not orange-brown to dark red-brown — and its spore walls are generally thinner. Furthermore, in *G. trufemii* the subtending hyphal pigmentation extends further below the spore base, both in spores formed in sporocarps or singly in the soil. Finally, the shape of the subtending hyphae is much more irregular in *G. brohultii* (Herrera-Peraza et al. 2003: this paper, Figs. 10–18).

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Two new species of *Pluteus* section *Celluloderma* from the Dominican RepublicALFREDO JUSTO¹*, ELISEO BATTISTIN² & CLAUDIO ANGELINI³¹Clark University, Biology Department, 950 Main St., Worcester 01610 MA USA²Natural History Museum, Corso Italia 63, Valdarno 36078 Italy³Via Tulipifero 9, Porcia 33080 Italy* CORRESPONDENCE TO: ajusto@clarku.edu

ABSTRACT— Two new species of *Pluteus* sect. *Celluloderma* collected in the Dominican Republic are described based on morphological and molecular (nrITS) characters. *Pluteus crenulatus* is characterized by the sulcate crenulate margin of the pileus, clavate cheilo- and caulocystidia, and absence of pleurocystidia. *Pluteus stenotrichus* is characterized by the presence of very narrow and internally septate cells in the pileipellis.

KEY WORDS— biodiversity, Caribbean, phylogeny, *Pluteaceae*, taxonomy

Introduction

The genus *Pluteus* Fr. (*Pluteaceae*, *Agaricales*) has been the focus of recent molecular phylogenetic studies (Menolli et al. 2010; Justo et al. 2011a,b), which essentially support the traditional subdivision of the genus in three sections (*Pluteus*, *Celluloderma* Fayod, and *Hispidoderma* Fayod) but with some rearrangements. Sect. *Celluloderma* still accommodates all species with non-metuloid hymenial cystidia and a pileipellis arranged as an hymeniderm or epithelium composed mostly of clavate or spheropedunculate elements (intermixed or not with elongated cells) but now, based on molecular data, also includes taxa with a cutis-type pileipellis (previously assigned to sect. *Villosi* Schreurs & Vellinga or *Hispidoderma*) or with distinct partial veil (previously assigned to *Chamaeota* (W.G. Sm.) Earle) (Menolli et al. 2010; Justo et al. 2011a,b; Vizzini & Ercole 2011).

Here we describe two new *Pluteus* species recently collected in the Dominican Republic. Morphological and molecular (nrITS) data support their inclusion in sect. *Celluloderma*. Both taxa are compared with morphologically similar species and their phylogenetic position is discussed.

Materials & methods

Collections were studied using standard procedures for morphological examination of *Pluteus* (e.g., Justo & Castro 2007, Minnis & Sundberg 2010). Descriptive morphological terms follow Vellinga (1988). The notation [30, 2, 1] indicates that measurements were made of 30 basidiospores from 2 basidiocarps in 1 collection. Color codes are from Munsell Soil-Color Charts (Munsell Color 2009). The following abbreviations are used in the descriptions: avl for average length, avw for average width, Q for the length/width quotient and avQ for average quotient.

Standard procedures for DNA isolation, PCR, and sequencing were applied (e.g., Justo et al. 2011a,b). The “nuclear ribosomal internal transcribed spacers 1 and 2 including the 5.8S region” is abbreviated as nrITS. The final dataset includes sect. *Celluloderma* nrITS sequences generated by Justo et al. (2011b), additional sequences generated by the first author, and sequences currently available in GenBank (mostly originated by O’Brien et al. 2005, Matheny et al. 2006, Midgley et al. 2007, Malysheva et al. 2009, Menolli et al. 2010). All accession numbers and sequence geographic origins are given in FIG. 1. Sequences were aligned using MAFFT version 6 (<http://mafft.cbrc.jp/alignment/server/>; Katoh & Toh 2008) with the Q-INS-i option, and alignments were manually corrected using MacClade 4.05 (Maddison & Maddison 2002) before deposition in TreeBASE (<http://purl.org/phylo/treebase/phyloids/study/TB2:S12007>). A Maximum Likelihood analysis was run using the RAXML servers with 100 rapid bootstrap replicates (<http://phylobench.vital-it.ch/raxml-bb/index.php>; Stamatakis et al. 2008). *Pluteus cervinus* (Schaeff.) P. Kumm. and *P. petasatus* (Fr.) Gillet (both sect. *Pluteus*) were used as outgroup taxa.

Results

The phylogenetic position of the new taxa described here is highlighted in FIG. 1 and discussed in detail under Taxonomy. Sequence identifications marked with “***” (which require further attention but fall outside the scope of this paper) are listed here to avoid causing further taxonomic confusion in GenBank: (i) “*Pluteus* cf. *nanus*” (HM562046) and “*P. nanus*” (JF908611) probably represent *P. cinereofuscus* J.E. Lange; (ii) “*Pluteus cinereofuscus*” (JF908616) is part of the *Pluteus nanus* (Pers.) P. Kumm. species complex; (iii) “*Pluteus cervinus*” (JF908623) represents a taxon close to *Pluteus ephebeus* (Fr.) Gillet; (iv) “*Pluteus galeroideus*” (JF908610, JF908609) probably represents *Pluteus chrysophlebius* (Berk. & M.A. Curtis) Sacc.; (v) “*Pluteus boudieri*” (JF908627) represents *Pluteus phlebophorus* (Ditmar) P. Kumm.; (vi) *Pluteus insidiosus* (JF908626) falls within collections morphologically assignable to *Pluteus thomsonii* (Berk. & Broome) Dennis.

FIGURE 1. Best tree from the Maximum Likelihood analysis of *Pluteus* sect. *Celluloderma*. Bootstrap values $\geq 70\%$ are shown on or below the branches. Root length has been reduced to facilitate graphical representation.

Taxonomy

Pluteus crenulatus Justo, Battistin & Angelini, **sp. nov.**

FIG. 2

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Similar to *Pluteus tucumanus*, also with a sulcate pileus with crenulate margin, but differing in broader spores, clavate cheilo- and caulocystidia, and absence of pleurocystidia

TYPE: Dominican Republic, Prov. Puerto Plata, Sosua, Unión, 17 Jan 2011, leg. C. Angelini, coll. AJ353 (Holotype MICH; GenBank nrITS JN603202. Isotype MCVE 27198).

ETYMOLOGY: *crenulatus* refers to the distinctive crenulate pileus margin.

PILEUS 20–40 mm, convex to plano-convex, with a broad umbo, deeply sulcate in the outer half; surface smooth or minutely granulose at center, granulose towards the margin; brown, darker at center [7.5YR 3/4, 4/6] and paler towards margin [7.5YR 5/6, 5/8, 6/6] where it alternates brown and whitish zones, not hygrophanous, margin distinctly crenulate, white. LAMELLAE crowded, free, ventricose; ≤ 4 mm broad; white when young, later pink with flocculose whitish edges. STIPE 30–50 $\times$ 2–4 mm, cylindrical, slightly broadened at base; white or white-cream; smooth or pruinose. CONTEXT in stipe and pileus white. SMELL indistinct. TASTE not recorded. SPORE PRINT not recorded, probably pink.

BASIDIOSPORES [30, 2, 1] 4.5–5.5(–6.0) $\times$ 3.9–5.0(–5.5) μm , $\text{avl} \times \text{avw} = 4.9 \times 4.2$ μm , $Q = 1.00$ –1.26, $\text{av}Q = 1.17$, globose to broadly ellipsoid. BASIDIA 20–35 $\times$ 9–12 μm , 4-spored, clavate or narrowly utriform. PLEUROCYSTIDIA absent. LAMELLAR EDGE sterile, completely covered with cystidia. CHELOCYSTIDIA 24–73 $\times$ 12–34 μm , (narrowly) clavate, a few narrowly utriform; colorless; with thin, smooth walls; crowded, forming a well-developed strip. PILEIPELLIS a hymeniderm with transitions towards and epithelium; individual elements 29–61 $\times$ 14–49 μm , mostly clavate or spheropedunculate, a few narrowly utriform, mucronate, or with basal septum; filled with brown intracellular pigment, evenly dissolved or more rarely with pigment condensations; with thin, smooth walls. STIPITPELLIS a cutis; hyphae 4–13 μm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. CAULOCYSTIDIA 38–51 $\times$ 17–31 μm , (narrowly) clavate, some multiseptate; hyaline or with brown intracellular pigment, some with additional incrusting pigment; with thin to slightly thickened (less than 1 μm), smooth walls; all over stipe surface in tightly packed clusters. CLAMP CONNECTIONS absent in all tissues.

HABITAT AND DISTRIBUTION—Gregarious, on a fallen log in broad-leaved forest, January. Known only from the type locality in the Dominican Republic.

COMMENTS—The deeply sulcate pileus with a crenulate margin is the most distinctive macroscopic feature of *P. crenulatus*. Microscopically, it is characterized by the clavate cheilo- and caulocystidia (FIG. 2) and the absence of pleurocystidia.

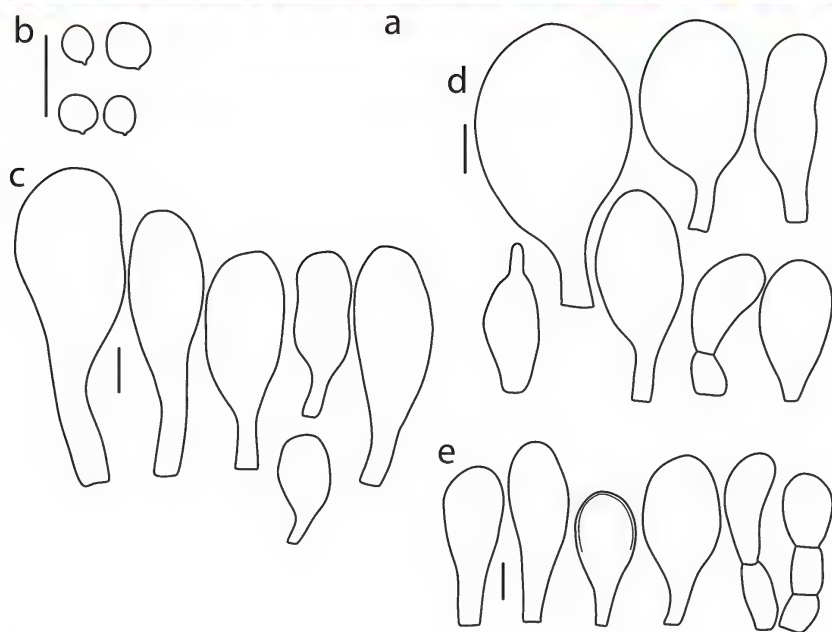


FIGURE 2. *Pluteus crenulatus* (holotype AJ353). a. Basidiocarps; b. Basidiospores; c. Cheilocystidia; d. Pileipellis elements; e. Caulocystidia. Scale bars = 10 μ m.

P. tucumanus is a South American species that also has a sulcate, appendiculate-crenate pileus (Singer & Digilio 1952; Singer 1956, 1958). The following observations are based on our examination of its isotype in MICH (Singer T921 Argentina, Prov. Tucumán, Rio de los Sosas, 18 Jan 1950):

Pluteus tucumanus Singer, Lilloa 25: 269. 1952 ["1951"].

FIG. 3

BASIDIOSPORES [30, 1, 1] $3.9\text{--}5.0 \times 2.7\text{--}4.0\ \mu\text{m}$, $\text{avl} \times \text{avw} = 4.5 \times 3.6\ \mu\text{m}$, $Q = 1.00\text{--}1.34\text{--}(1.69)$, $\text{av}Q = 1.26$, globose to ellipsoid, very rarely oblong. BASIDIA $25\text{--}31 \times 7\text{--}10\ \mu\text{m}$, 4-spored, narrowly utriform. PLEUROCYSTIDIA $40\text{--}53 \times 18\text{--}22\ \mu\text{m}$, (narrowly) utriform with a distinct pedicel; colorless; with thin, smooth walls; scarce and scattered, present all over lamellar sides. LAMELLAR EDGE sterile, completely covered with cystidia. CHELOCYSTIDIA $29\text{--}52 \times 12\text{--}32\ \mu\text{m}$, (narrowly) utriform or broadly fusiform, a few narrowly clavate; colorless; with thin, smooth walls; crowded, forming a well-developed strip. Pileipellis an hymeniderm with transitions towards and epithelium; individual elements $38\text{--}49 \times 21\text{--}32\ \mu\text{m}$, mostly clavate or spheropedunculate; filled with brown intracellular pigment, evenly dissolved or more rarely with pigment condensations; with thin, smooth walls. STIPITPELLIS a cutis; hyphae $5\text{--}13\ \mu\text{m}$ wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. CAULOCYSTIDIA $38\text{--}51 \times 17\text{--}31\ \mu\text{m}$, (narrowly) clavate to (narrowly) utriform; hyaline or with brown intracellular pigment; with thin, smooth walls; all over stipe surface, scattered or in clusters. CLAMP-CONNECTIONS absent in all tissues.

Although the differences between *P. crenulatus* and *P. tucumanus* are subtle (the latter distinguished by narrower spores, presence of pleurocystidia, and differently shaped cheilo- and caulocystidia), such subtle variations have proved critical in separating species in sect. *Celluloderma* (Justo et al. 2011c).

Singer (1958) placed *Pluteus fallax* Singer and *P. iguazuensis* Singer into the same morphological group (stirps *Tucumanus*) as *P. tucumanus*. We were unable to examine either species, but based on Singer's (1958) descriptions both taxa differ from *P. crenulatus* in (among other characters) the non-crenulate pileus margin and presence of pleurocystidia. Singer also emphasized the presence of pigment condensations in the pileipellis elements of *P. tucumanus* and the absence of such condensations in *P. fallax* and *P. iguazuensis*. We observed pigment condensations in both *P. crenulatus* and *P. tucumanus*, but the taxonomic significance of this feature needs further study.

Macroscopically, *P. crenulatus* does not resemble any species in sect. *Celluloderma* with very rare to absent pleurocystidia — e.g., *P. diettrichii* Bres., *P. insidiosus* Vellinga & Schreurs, *P. poliocnemis* Kühner — which also differ microscopically in spore and cystidia shape and size (see Kühner & Romagnesi (1956) and Vellinga (1990) for full descriptions). *Pluteus thomsonii*

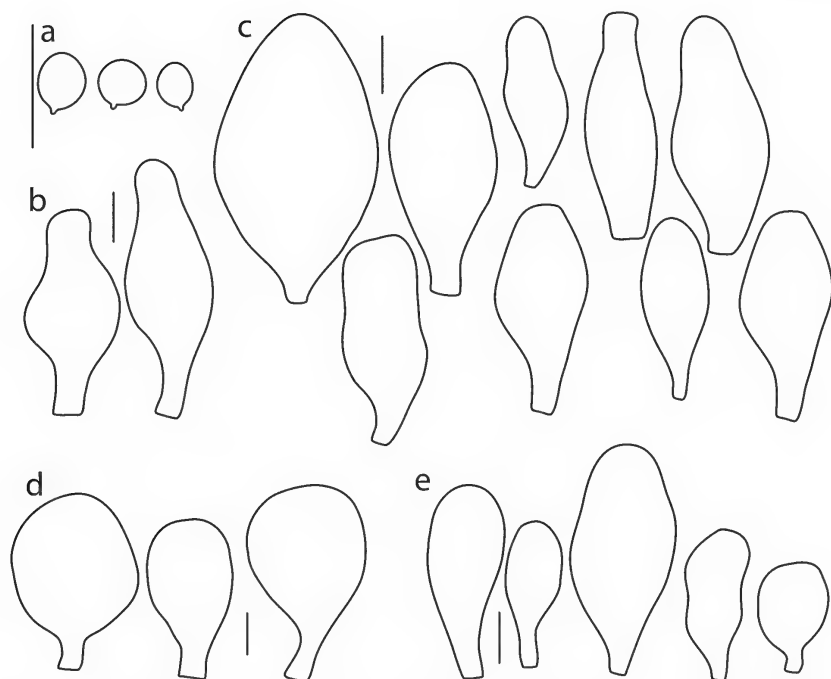


FIGURE 3. *Pluteus tucumanus* (isotype, Singer T921). a. Basidiospores; b. Pleurocystidia; c. Cheilocystidia; d. Pileipellis elements; e. Caulocystidia. Scale bars = 10 μ m.

and *P. seticeps* (G.F. Atk.) Singer, which sometimes have very rare or absent pleurocystidia, differ from *P. crenulatus* by the “*Mixtini*-type” pileipellis (see Minnis & Sundberg (2010) and Vellinga (1990) for full descriptions).

Phylogenetically, *P. crenulatus* appears somewhat isolated as sister to all taxa in sect. *Celluloderma* except *P. diettrichii* Bres. (FIG. 1). This placement receives no statistical support but suggests that *P. crenulatus* is not closely related to any of the major lineages presently recognized in sect. *Celluloderma* (Justo et al. 2011b).

***Pluteus stenotrichus* Justo, Battistin & Angelini, sp. nov.**

FIG. 4

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Characterized by a “*Mixtini*-type” pileipellis with very narrow, internally septate cylindrical elements.

TYPE: Dominican Republic, Prov. Puerto Plata, Sosua, El Castillo, 16 Jan 2011, leg. C. Angelini, coll. AJ352 (Holotype MICH; GenBank nrITS JN603201. Isotype MCVE 27197).

ETYMOLOGY: *stenotrichus* (= “with narrow hairs”) refers to the very unusual type of pileipellis cells.

PILEUS (20–)25–50(–60) mm, convex to plano-convex, with or without a low broad umbro; surface distinctly rugose-venose at and around center, radially areolate-rimose in the outer half, exposing the white context; brown, darker at center [7.5YR 2.5/2, 2.5/3, 3/2, 3/3], paler around center [7.5YR 4/6, 5/6, 5/8] and much paler at margin [7.5YR 7/4, 7/6, 7/8], not hygrophanous; margin rimose but not striate or sulcate. LAMELLAE crowded, free, ventricose; ≤ 7 mm broad; white or cream when young, later pink [5YR 8/3, 8/4], with concolorous or paler edges. STIPE 30–60 $\times$ 4–6 mm, cylindrical, slightly broadened at base; white or white-cream; smooth or slightly pruinose. CONTEXT in stipe and pileus white. SMELL indistinct. TASTE not recorded. SPORE PRINT not recorded, probably pink.

BASIDIOSPORES [30, 2, 1] 4.9–7.0 $\times$ 4.5–5.7 μm , $\text{avl} \times \text{avw} = 5.6 \times 5.1 \mu\text{m}$, $Q = 1.00$ –1.18, $\text{av}Q = 1.11$, globose to broadly ellipsoid. BASIDIA 25–34 $\times$ 7.5–10 μm , 4-spored, clavate or narrowly utriform. PLEUROCYSTIDIA 34–83 $\times$ 14–32 μm , (narrowly) utriform or lageniform, a few broadly conical with long pedicel; colorless; with thin, smooth walls; relatively common, scattered over lamellar sides. LAMELLAR EDGE sterile, completely covered with cystidia. CHELOCYSTIDIA 25–50(–65) $\times$ 14–20(–25) μm , (narrowly) clavate, spheropedunculate, some broadly fusiform, some with basal septum; colorless; with thin, smooth walls; crowded, forming a well-developed strip. PILEIPELLIS a hymeniderm with transitions towards and epithelium, composed of three types of elements: (i) globose, clavate or spheropedunculate 31–76 $\times$ 22–60 μm ; (ii) narrowly clavate or lageniform 53–88 $\times$ 15–29 μm ; (iii) very narrow elongated elements, 60–123 $\times$ 7–10(–12) μm , cylindrical or flexuous, with 0–4 internal septa, usually arising from a basal element $\leq 15 \mu\text{m}$ wide; all elements filled with brown intracellular pigment, more rarely hyaline; with thin, smooth walls. STIPITPELLIS a cutis; hyphae 5–15 μm wide, cylindrical, colorless or with brown pigment; with thin, smooth walls. CAULOCYSTIDIA 31–63 $\times$ 12–24 μm , (narrowly) clavate, narrowly utriform or conical with basal septum; hyaline or with brown intracellular pigment; with thin, smooth walls; scattered all over stipe surface, isolated or in loosely arranged clusters. CLAMP CONNECTIONS absent in all tissues.

HABITAT AND DISTRIBUTION—Gregarious, apparently terrestrial (among woody remnants and organic matter) in broad-leaved forest, January. Known only from the type locality in the Dominican Republic.

COMMENTS—The very narrow and internally septate elements in the pileipellis (FIG. 4) are diagnostic for *P. stenotrichus*. Taxa with both clavate-spheropedunculate and elongated elements in the pileipellis have been traditionally classified in subsect. *Mixtini* Singer, but molecular data suggest that elongated elements in the pileipellis have evolved several times within sect. *Celluloderma*, rendering subsect. *Mixtini* artificial (Justo et al. 2011a,

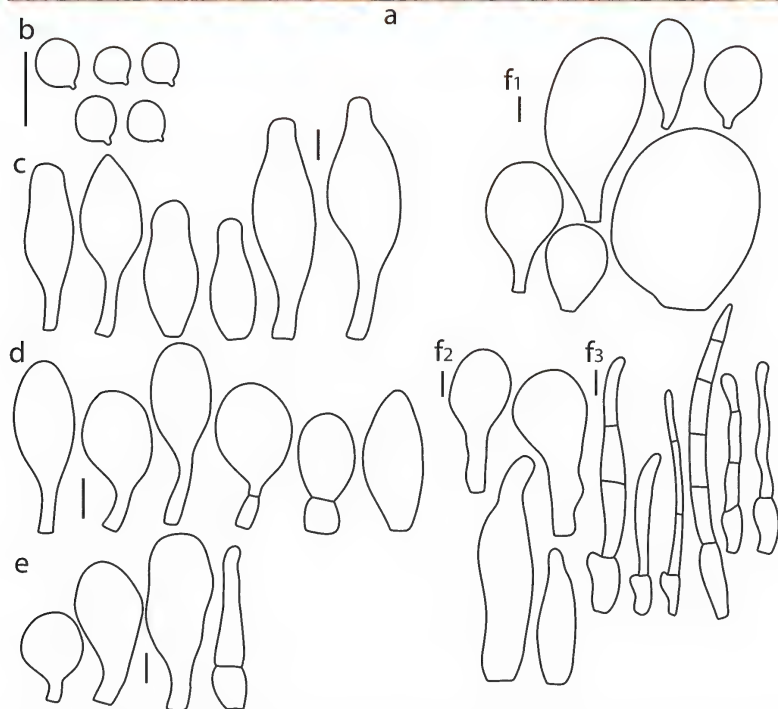


FIGURE 4. *Pluteus stenotrichus* (holotype AJ352). a. Basidiocarps; b. Basidiospores; c. Pleurocystidia; d. Cheilocystidia; e. Caulocystidia; f. Pileipellis elements – f1 clavate and spheropedunculate, f2 narrowly clavate and lageniform, f3 very narrow and internally septate. Scale bars = 10 μ m.

Fig. 1). An extensive literature search and comparison with all species described with a “*Mixtini*-type” pileipellis (e.g. Horak 1964, 2008; Horak & Heinemann 1978; Minnis & Sundberg 2010; Orton 1986; Pegler 1977, 1986; Singer 1956, 1958; Vellinga 1990; see also Justo et al. 2011a,b,c for additional taxonomic references) found none with this type of septate elongated element.

Pluteus stenotrichus is not closely related to any previously sequenced species with a “*Mixtini*-type” pileipellis (Fig. 1) and its position in the *romellii/aurantiorugosus* group is rather unexpected based on morphology. *Pluteus aurantiorugosus* (Trog) Sacc., *P. romellii* (Britzelm.) Sacc., and taxa in the species complex around *P. aureovenatus* Menolli & Capelari all lack any elongated pileipellis elements and are characterized in part by the bright yellow or orange-red colors of the pileus and/or stipe. The closest relative of *P. stenotrichus* is an undescribed species from Brazil (nrITS HM562173, collection SP394383) but that taxon differs in the (among other characters) absence of elongated pileipellis elements and almost total absence of hymenial cystidia (N. Menolli Jr. & M. Capelari, pers. comm.).

The narrow septate elements in the pileipellis of *P. crenulatus* are reminiscent of those typically exhibited by some members of sect. *Hispidoderma* (e.g., *P. exiguus* (Pat.) Sacc.). However the general organization of the pileipellis differs in those taxa and lacks the well-developed hymeniform layer made up of clavate or spheropedunculate elements that is typical of *P. stenotrichus* and the other species with “*Mixtini*-type” pileipellis in sect. *Celluloderma*.

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The lichen genus *Chapsa* (Graphidaceae) in India

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ABSTRACT — This paper provides a detailed account of the 16 species of *Chapsa* recorded in India. *Chapsa patens* and *C. platycarpella* are new records for the Indian lichen biota.

KEY WORDS — thelotremoid, distribution, identification key, Western Ghats

Introduction

Until recently the thelotremataceous lichen biota was limited in India to *Chroodiscus*, *Diploschistes*, *Leptotrema*, *Myriotrema*, *Ocellularia*, and *Thelotrema* (Awasthi 1991, 2000). Based on recent revisions (Frisch et al. 2006, Mangold et al. 2009, Rivas Plata et al. 2010), Singh & Sinha (2010) cited 130 taxa representing 13 genera in the *Thelotremataceae* Stizenb. for India. However, recent phylogenetic studies show that taxa previously classified in *Thelotremataceae* do not form a separate lineage but are nested within *Graphidaceae*. The *Graphidaceae*, containing the now-synonymous *Thelotremataceae* (Mangold et al. 2008), comprises the largest lichen family in India, where it is represented by 428 species and 35 genera.

Massalongo introduced *Chapsa* in 1860, a genus resurrected recently by Frisch et al. (2006) to incorporate species earlier classified in *Thelotrema*, *Chroodiscus*, *Ocellularia*, and *Myriotrema*. *Chapsa* is characterized by a thin, corticolous, endoperidermal to epiperidermal thallus, chroodiscoid ascomata with a fused to $\pm$ free proper exciple, and periphysoids (Mangold et al. 2009). The closely related or similar *Acanthotrema*, *Chroodiscus*, and *Reimnitzia* differ mainly in spiny paraphysis tips, lack of periphysoids, and isidiate thallus with columnar crystalline layer, respectively. Moreover, *Chroodiscus* is typically foliicolous. *Thelotrema* and *Topeliopsis* differ mainly by lacking distinctly chroodiscoid ascomata. However, several species belonging to the last two genera require further investigation for proper delimitation and placement.

Due to their selective and preferred habitat, most members of thelotremoid *Graphidaceae* are restricted to lower altitudes in tropical to subtropical regions and thus inadequately understood (Hale 1974, 1978, 1981). As a group, thelotremoid lichens represent a significant component of corticolous rainforest microlichen biota, with some extending into temperate regimes (Santesson 1952; Sipman & Harris 1989; Rivas Plata et al. 2008; Purvis et al. 1995). *Chapsa* is pantropical or subtropical but rarely temperate. Generally, it occurs below 1000 m and favors shaded microhabitats in rather disturbed old growth secondary forests (Rivas Plata et al. 2008). *Chapsa* flourishes in the southern and eastern regions of India, with some species found at altitudes of more than 2000 m. The dense moist rain forests of the eastern Himalayas and the evergreen forests of Western Ghats provide a suitable habitat for its growth. Most species of *Chapsa* grow on rough bark, thus indicating a particular type of forest community predominant in moist humid regions. The species generally acquire the same colour pattern as the substratum so that in the field many are difficult to distinguish from bark. The tree bark characteristics appear to influence the distribution patterns of the taxa in tree communities. Lichens growing on striate bark concentrate in deep furrows, with their ascomata emerging from cracks, while in many species the thallus surface mirrors inflated bark. Most taxa, however, grow on flaking bark with only ascomata protruding out of the thallus.

The ascomatal features and chemical substances play a vital role in differentiating the different thelotremoid groups. Based on morphology, the ascomata may be of the chroodiscoid, fissurinoid, leprocarpoid, platycarpoid, lamelloid, scabioid, or topeleipsoid types. However, except for *C. platycarpa* and *C. recurva*, all Indian specimens lack secondary compounds.

Sixteen species of *Chapsa* are currently known from India. The evergreen forests in the Western Ghats represented by nine species was the most diverse, with six species known from the tropical rain forests of the eastern Himalayas and four species known from the Andaman and Nicobar Islands. One report of *Chapsa dilatata* (Müll. Arg.) Kalb from Arunachal Pradesh (Dubey et al. 2008, as *Thelotrema dilatatum*) was based on a misdetermined specimen that represents a *Myriotrema* species. All accurately identified *Chapsa* species recorded from India are presented here with brief descriptions.

Materials & methods

Material preserved in the herbarium of the National Botanical Research Institute (IWG) was examined morphologically, anatomically, and chemically. Thin hand-cut sections of apothecia and thallus mounted in tap water, cotton blue, 5% KOH, and iodine solution were observed under a compound microscope (LEICA DM 500). Chemical spot tests and TLC (using solvent system A) were conducted according to Orange et al. (2001).

Taxonomic descriptions

Chapsa alborosella (Nyl.) Frisch, Biblioth. Lichenol. 92: 90, 2006.

The species is characterized by an ecorticate, pale olive green thallus lacking lichen compounds, round to angular or shortly elongate pale brown apothecia level with the thallus, 8-spored asci and hyaline, transversely septate, fusiform to clavate or oblong, I– ascospores.

Chapsa platycarpella differs from the closely related *C. alborosella* in having a corticate thallus and a pale to blackish apothecial disc. Patwardhan & Kulkarni (1977a) and Nagarkar et al. (1988) reported this species from south India as *Ocellularia alborosella*. The species is common in tropical evergreen forest of Kerala in the Western Ghats.

“*Chapsa hiata*” (Hale), ined.

Known from evergreen forests in the southern part of India, this species is well characterized by an ecorticate thallus lacking lichen substances, wide and open apothecia with recurved margins, free proper exciple, 4–8-spored asci, and small hyaline muriform I– ascospores measuring $14\text{--}16 \times 4\text{--}7\mu\text{m}$.

Patwardhan et al. (1985) reported it from Karnataka as *Thelotrema hiatum*. Similar to *Chapsa leprocarpoides*, *C. velata* and *C. pseudophlyctis* in lacking thallus compounds, the species differs in having small ascospores. The latter two species also differ in having a fused proper exciple.

Chapsa indica A. Massal., Atti I. R. Ist. Veneto Sci. Lett. Arti, Ser. 3, 5: 257, 1860.

This taxon is characterised by an ecorticate brownish-white to olive-grey thallus lacking lichen compounds, rounded to angular or shortly elongated and slightly branched apothecia that are level with the thallus, 6–8-spored asci, and hyaline transversely septate oblong-fusiform ascospores.

Patwardhan & Nagarkar (1980) and Nagarkar et al. (1988) first reported the species from India (as *Ocellularia pycnophragmia* (Nyl.) Zahlbr.) from tropical rain forests of Andaman and Nicobar, Assam, and Meghalaya. Similar in having an ecorticate whitish (-brownish) to olive-grey thallus, *C. leprocarpa* and *C. pseudophlyctis* differ from *C. indica* in having muriform ascospores.

Chapsa laceratula (Müll. Arg.) Rivas Plata & Lücking, Lichenologist 42: 183, 2010.

The species exhibits a pale to dark olive-green to olive brown, corticate surface lacking secondary metabolites, round to irregular, perithecioid to apothecioid apothecia with lacerate margins, 1–2(–4)-spored asci, and hyaline, muriform, fusiform ascospores.

Patwardhan & Kulkarni (1977a) reported *Chapsa laceratula* (as *Thelotrema laceratulum*) from Karnataka, Kerala, Maharashtra, and Tamil Nadu in southern India. The scabioid apothecia with vertically layered excipulum and

≤4-spored asci are the major distinguishing characters of *C. laceratula*, which morphologically may be confused with *Topeliopsis* species. It is generally distributed at lower elevation and growing luxuriantly in evergreen forests of Western Ghats.

SPECIMENS EXAMINED: **INDIA. KARNATAKA:** Central Western Ghat, Shimoga district, 3 km before Agumbe Ghat, from Koppa to Agumbe, alt. 649 m, on bark in evergreen forest, 14.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19730M/A (LWG); Uttarkannad, Sharavathi Ghat, near Gersoppa, alt. 718 m, on bark in evergreen forest, 16.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19744A-1, 19744B, 1944C-2, 19744I, 19745, 19745A-3, 19745C (LWG); **TAMIL NADU:** Madurai district, High ways Vattapara (Meghamali), alt. 1580 m, on fallen twigs, 22.03.1999, S. Nayaka 92(LWG).

Chapsa lepriurii (Mont.) Frisch, Biblioth. Lichenol. 92: 105, 2006.

The species is characterized by a brown-olive, cartilaginous thallus lacking secondary compounds, rather small, rounded to slightly elongated or branched apothecia level with the thallus, 8-spored asci, and transversely septate brown oblong to ellipsoidal or clavate ascospores.

Chapsa lepriurii has been reported from Karnataka, Kerala, and Tamil Nadu in southern India (Awasthi 1991, as *Thelotrema lepriurii*; Singh & Sinha 2010, as *T. lepriurii*). The brown-spored similar species, *C. platycarpa*, differs in producing lichen compounds and a double margin.

Chapsa leprocarpa (Nyl.) Frisch, Biblioth. Lichenol. 92: 108, 2006.

The taxon exhibits a pale to dark olive-grey thallus lacking lichen compounds, large chroodiscoid apothecia with a lobed and recurved margin, pale brown to blackish but heavily white pruinose disc, 1-spored asci, and muriform ascospores.

Awasthi (1991) first recorded *C. leprocarpa* (as *Thelotrema colobicum*) from Andaman and Nicobar Islands. *Chapsa leprocarpa* inhabits lowland and submontane regions in the Western Ghats.

SPECIMENS EXAMINED: **INDIA. KERALA:** Idukki district, I.C.R.I. campus, Myladumpara, alt. ca. 1200 m, on bark of tree, 01.03.1984, D.D. Awasthi & G. Awasthi 84.88 (LWG-LWU); on way Myladumpara to Munnar, Santhampara area, alt. ca. 1200 m, on bark of tree, 02.03.1984, D.D. Awasthi & G. Awasthi 84.179 (LWG-LWU); Thikkady, Periyar Tiger Reserve, Sakunthalakadu, on bark, 23.03.2006, B. Haridas 06-009615/B (LWG).

Chapsa leprocarpoides (Hale) Cáceres & Lücking, Libri Bot. 22: 52, 2007.

The species is ecorticate, pale olive to fawn coloured, lacking lichen substances, without compact surface, usually reflecting bark through the matted thallus surface; it has immersed to semi-immersed, rounded to angular apothecia with a flesh-coloured pruinose disc and low jagged to lobed margins, a pale to brownish or hyaline free proper exciple, 8-spored asci, and hyaline muriform ellipsoidal to oval ascospores.

Chapsa leprocarpoides was collected from evergreen tropical forests of Karnataka on rough tree barks at lower altitude. The ecorticate "*Chapsa hiata*" differs in having rather small ascospores while *C. velata* and *C. pseudophlyctis* have comparatively larger ascospores and a fused proper exciple.

SPECIMENS EXAMINED: INDIA. KARNATAKA: Chikmagalur district, Chamudi Ghat, Kuvettu, alt. 104 m, on bark in evergreen forest, 15.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19739R; Uttar Kannad, Sharavati Ghat, near Gersoppa, alt. 718 m, on bark in evergreen forest, 16.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19744J (LWG); Shimoga district, Sagar to Talguppa, Ulanahalli, alt. 697 m, on bark in evergreen forest, 16.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19742E, 19742E-1(LWG); Central Western Ghat, Shimoga district, near Jog fall, Kargal, alt. 644 m, 16.01.2008, H.T. Lumbsch, D.K. Upreti, P.K. Divakar & J. Tandon 19742B-2, 19743O-1 (LWG).

Chapsa meghalayensis (Patw. & Nagarkar) Lumbsch & Divakar, Lichenologist 42: 183, 2010.

This taxon is characterized by its ashy-white, ecorticate, granular thallus lacking lichen compounds, immersed and chroodiscoid apothecia with recurved margins, lacerate exciple, 2-spored asci, and small muriform brown ascospores measuring $27\text{--}46 \times 11\text{--}16 \mu\text{m}$.

First described from Meghalaya by Patwardhan & Nagarkar (1980) as *Leptotrema meghalayense*, the species was later transferred to *Myriotrema* by Awasthi (1991). *Chapsa meghalayensis* is endemic to India and collected from evergreen forests of northeast India.

Chapsa patens (Nyl.) Frisch, Biblioth. Lichenol. 92: 111, 2006. PL. 1A

Thallus corticolous, epiperidermal, ecorticate, dark olivaceous grey to dark grey or blackish, continuous, $\pm$ cracked due to bark texture, uneven, bark reflecting through thallus. Prothallus indistinct. Photobiont layer well developed, up to $50 \mu\text{m}$ thick. Medulla endoperidermal. Apothecia scattered, sometimes grouped in two, immersed to level with thallus, mostly rounded to slightly angular or irregular in shape, up to 2.0 mm in diam. Margin raised, lobed to $\pm$ continuous, mostly straight to recurved later, brownish to faintly crystalline pruinose inner side. Disc reddish-brown, exposed, covered by thick pruina. Proper exciple cupular, pale to hyaline, sometimes brownish, up to $40\text{--}80 \mu\text{m}$ thick laterally. Periphysoides indistinct to $30 \mu\text{m}$ long, inclined towards epihymenium. Epihymenium $10\text{--}15 \mu\text{m}$ high, slightly brownish, granular. Hymenium, clear, $130\text{--}140 \mu\text{m}$ high. Paraphyses entangled, simple, $\leq 1.5 \mu\text{m}$ thick, apically thickened, $\pm$ dendroid branched, adspersed with grayish granules. Ascus, 1-spored, clavate, $90\text{--}130 \times 25\text{--}40 \mu\text{m}$. Ascospores hyaline, densely muriform, with rounded ends, $80\text{--}125 \times 20\text{--}35 \mu\text{m}$, I-.

CHEMISTRY: K-, PD-, C-; no lichen compound detected in TLC.

ECOLOGY & DISTRIBUTION: The species is a new record to India reported from tropical rain forests of the Eastern Himalayas where it is common in open,

canopied old forests with large tree trunks and generally found at 1700–2500 m. Widely distributed and reported from Africa, Japan, and Sri Lanka.

SPECIMEN EXAMINED: INDIA. WEST BENGAL: Eastern Himalaya, Darjeeling district, Tiger hill, north face of the hill, alt. ca. 2500 m, on bark of tree, 05.03.1967, D.D. Awasthi & M.R. Agarwal 67.21 (LWG-LWU).

REMARKS: *Chapsa leprocarpa*, which also lacks lichen substances, differs from *C. patens* in having a slightly lower hymenium and smaller ascospores and grows at lower altitudes (1000–1200 m). The Indian specimen of *C. patens* exhibits a dirty grey to dark olivaceous thallus colour hardly distinguished from the bark, while *C. leprocarpa* has a pale-green to slightly greyish-green epiperidermal thallus. However, the hymenium and ascospores of the Indian sample are intermediate in size and closer to *C. patens* than *C. leprocarpa* and its distribution and thallus and apothecial morphology also agree well with the diagnostic features of *C. patens*. Hale (1981) and Matsumoto (2000) both described *C. patens* as a stictic acid-producing species that after re-investigation were shown to lack these substances. The records from Japan and Sri Lanka should be re-examined (Frisch et al. 2006).

Chapsa platycarpa (Tuck.) Frisch, Biblioth. Lichenol. 92: 113, 2006.

The species is recognized by the dark olive-green to olive-brown or pale yellowish-brown thallus containing the stictic acid chemosyndrome, a rounded to slightly irregular apothecioid to chroodiscoid shape in older material, erumpent apothecia with double margins, 8-spored asci, and transversely septate ellipsoidal to fusiform brown ascospores.

Earlier placed in the neglected genus *Phaeotrema* due to its transversely septate brown ascospores, *Chapsa platycarpa* was reported from India (Kerala) as *P. platycarpum* (Patwardhan & Kulkarni 1977a). The similar *C. leprieurii* lacks stictic acid as a major thallus compound.

Chapsa platycarpella (Vain.) Frisch, Biblioth. Lichenol. 92: 118, 2006. PL. 1B

Thallus corticolous, endoperidermal, olive-green to pale-green, continuous, shiny, compact, cartilaginous, uneven to warty. Prothallus indistinct to brownish. Medulla white, indistinct, endoperidermal. Phenocortex well developed, dense, 20–30 µm thick. Photobiont layer 20–35 µm thick, with inclusion of calcium oxalate crystals, largely endoperidermal. Apothecia scattered to aggregate in groups, immersed to level with thallus, round to mostly angular or irregular in shape, ≤1.0 mm in diam. Margin thin, fissured to lobed, recurved, and yellowish to fawn with felty white pruinose to crystalline inner surface. Disc pale to blackish, distinctly white pruinose. Proper exciple cupular, pale to hyaline, 6–10 µm wide. Periphysoides indistinct to 25 µm long, inclined towards epihymenium. Epihymenium 5–8 µm high, granular, unpigmented

to crystalline. Hymenium clear, 55–95 μm high. Paraphyses simple, straight, ≤ 2.0 μm thick, tips agglutinate with fine greyish to brownish granules. Ascus 8-spored, clavate, 45–90 $\times$ 6–9 μm . Ascospores, hyaline, transversely 3–5 septate, fusiform with acute end cells, 12–16 $\times$ 3–5 μm , 1–.

CHEMISTRY: K–, PD–, C–; no lichen compounds detected in TLC.

ECOLOGY & DISTRIBUTION: Known to grow on smooth bark of young trees in lowland rainforests and coastal forests in shade as well as on exposed surfaces. In India, reported on trees in evergreen tropical forest in Kerala.

SPECIMEN EXAMINED: INDIA. KERALA: Idukki, Adimali Forest Range, Thondi Kappu, on *Myristica beddomei*, 15.02.2006, B. Haridas 06–009598(LWG).

REMARKS: *Chapsa platycarpella*, which is considered a separate species by Frisch et al. (2006), is otherwise treated as a synonym of *C. alborosella*. The species is well characterized and separated from the latter in having a cartilaginous thallus, thick phenocortex, and loosely aggregated apothecia with blackish discs covered by bluish-white pruina.

Recently, Rivas Plata et al. (2010) placed the species in synonymy with *C. astroidea* (Berk. & Broome) Cáceres & Lücking; however following Frisch (2006) with adequate distinguishing characteristics the species is accepted here as a new record for India.

Chapsa pseudoexanthismocarpa (Patw. & C.R. Kulk.) Rivas Plata & Lücking, Lichenologist 42: 183, 2010.

The taxon is characterized by a pale yellowish to greenish brown or pale olive thallus lacking secondary compounds, apothecia that are rounded to somewhat irregular and sometimes perithecioid, erumpent, immersed to distinctly emergent, 4–8-spored asci, and hyaline transversely septate bacilliform-fusiform ascospores.

Patwardhan & Kulkarni (1977b) described the species from southern India (Kerala) in *Ocellularia*. Larger ascospores readily distinguish *C. pseudoexanthismocarpa* from *C. indica*. Other characters include the rough epiperidermal thallus, lepadinoid apothecia, and lacerate exciple.

Chapsa pseudophlyctis (Nyl.) Frisch, Biblioth. Lichenol. 92: 120, 2006.

The species is characterized by its white to whitish, whitish-grey to pale-grey thallus lacking secondary compounds, round to angular apothecia with jagged, often eroded margins having a white crystalline inner surface, 1–6-spored asci, and hyaline muriform ascospores.

Chapsa pseudophlyctis may sometimes be confused with *C. leprocarpa*, which can be distinguished by asci consistently bearing a single larger ascospore. Jagadeesh Ram & Sinha (2009) first reported *C. pseudophlyctis* as new to India from Meghalaya and Sikkim.

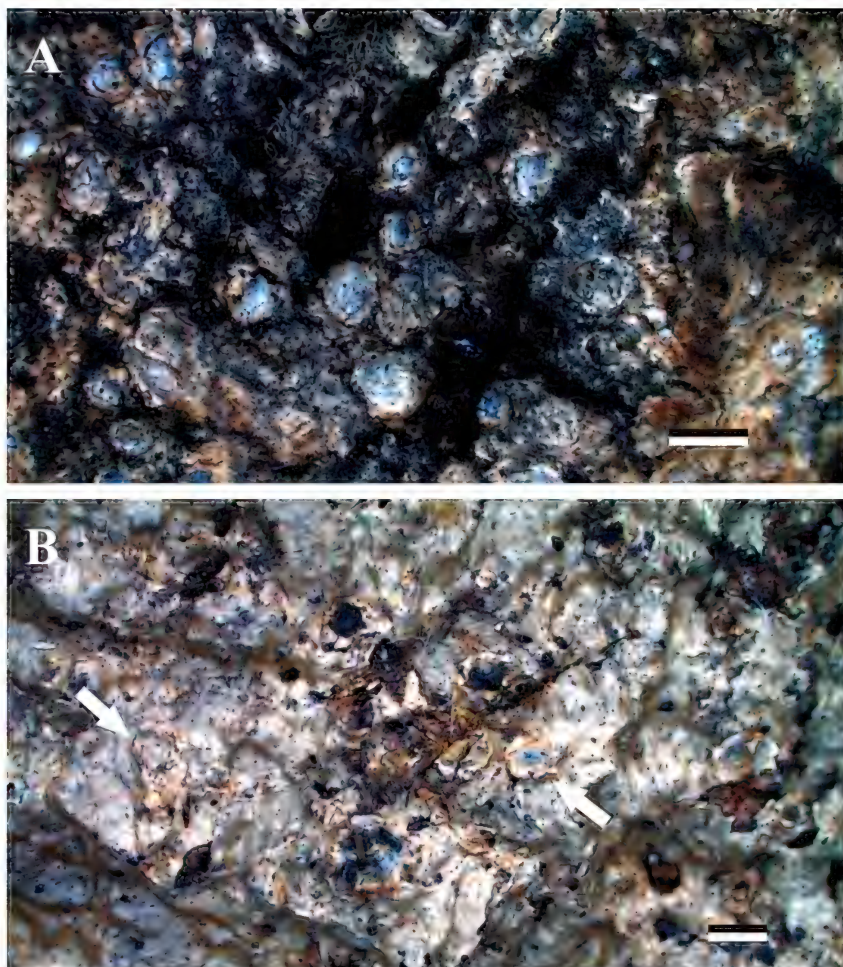


PLATE. 1. New records of *Chapsa* from India.
A. *Chapsa patens*. B. *Chapsa platycarpa* (note the apothecia).
Scale bars: A = 2 mm; B = 1 mm.

Chapsa recurva (G. Salisb.) Frisch, Biblioth. Lichenol. 92: 95, 2006.

Chapsa recurva and *C. platycarpa* are the only two *Chapsa* species that produce stictic acid as a major secondary compound (Rivas Plata et al. 2010). Nagarkar et al. (1986) reported the species (as *Thelotrema recurvum*) from Andaman & Nicobar Islands, but noted the absence of secondary compounds. Other major distinguishing characters include the corticate whitish grey smooth thallus, numerous solitary round to elongated semi-emergent chroodiscoid apothecia, and hyaline muriform ascospores.

“*Chapsa stellata*” (Hale), ined.

The taxon is recognized by the greenish, glaucous, continuous, mostly epiperidermal thallus lacking lichen substances, dispersed, slightly immersed chroodiscoid apothecia having a white pruinose disc and recurved margins, a $\pm$ fused brown proper exciple, 1-spored asci, and ellipsoidal olivaceous to brownish muriform ascospores, $88\text{--}135 \times 22\text{--}30\text{ }\mu\text{m}$. Patwardhan & Nagarkar (1980) reported the species (as *Leptotrema stellatum*) from Meghalaya in the eastern Himalayas.

***Chapsa velata* (Nyl.) Cáceres & Lücking, Libri Bot. 22: 54, 2007.**

The species is characterized by the ashy-white smooth partly hypophloeodal thallus lacking lichen substances, semi-emergent chroodiscoid apothecia with erect to recurved margins, fused to free brown proper exciple, hyaline hymenium, 4–8-spored asci (1–2-spored according to Patwardhan & Nagarkar 1980), and hyaline ellipsoidal muriform ascospores. The species was recorded from the Andaman Islands (Awasthi 1991, as *Thelotrema velatum*) and Nagaland (Patwardhan & Nagarkar 1980, as *T. velatum*).

Key to *Chapsa* species recorded from India

- 1a. Ascospores transversely septate 2
- 1b. Ascospores (sub-) muriform 7
- 2a. Ascospores hyaline 3
- 2b. Ascospores brown 6
- 3a. Ascospores $> 40\text{ }\mu\text{m}$ 4
- 3b. Ascospores $< 40\text{ }\mu\text{m}$ 5
- 4a. Ascospores (40–) 70–120 μm long*C. indica*
- 4b. Ascospores 100–200 μm long*C. pseudoexanthismocarpa*
- 5a. Ascospores 12–16 μm long, 4–6-septate, corticate*C. platycarpella*
- 5b. Ascospores 17–22 μm long, 5–9-septate, ecorticate*C. alborosella*
- 6a. Stictic acid present, proper exciple free (double margin),
 apothecial disc grey-brown*C. platycarpa*
- 6b. Lichen substances absent, proper exciple fused,
 disc pale brown to greyish*C. leprieurii*
- 7a. Ascospores hyaline 8
- 7b. Ascospores brown 5
- 8a. Ascospores $\leq 60\text{ }\mu\text{m}$ long 9
- 8b. Ascospores $> 60\text{ }\mu\text{m}$ long 13
- 9a. Ascospores 12–15 μm long, lichen substances absent“*C. hiata*”
- 9b. Ascospores $> 15\text{ }\mu\text{m}$ long, chemistry variable 10

- 10a. Stictic acid present *C. recurva*
 10b. Lichen substances absent 11
 11a. Proper exciple free, apothecia often aggregate,
 ascospores $30-40 \times 10-12 \mu\text{m}$ *C. leprocarpoides*
 11b. Proper exciple fused apothecia solitary, ascospores $30-60 \times 12-20 \mu\text{m}$ 12
 12a. Thallus ashy-white, apothecial disc dark-grey, thinly pruinose
 with bluish tinge *C. velata*
 12b. Thallus pale-olive, apothecial disc pale brown,
 distinctly white pruinose *C. pseudophlyctis*
 13a. Thallus with prosoplectenchymatous cortex, proper exciple layered *C. laceratula*
 13b. Thallus ecorticate 14
 14a. Ascospores $\leq 125 \mu\text{m}$ long *C. patens*
 14b. Ascospores $\leq 90 \mu\text{m}$ long *C. leprocarpa*
 15a. Ascospores $27-46 \times 11-16 \mu\text{m}$ *C. meghalayensis*
 15b. Ascospores $88-135 \times 22-30 \mu\text{m}$ "*C. stellata*"

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A new species of *Inonotus* (Hymenochaetaceae) and *Trametes cingulata* (Polyporaceae) newly recorded from Brazil

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ABSTRACT — Two interesting species found during a survey of polypore fungi in northwestern São Paulo State, Southeast Brazil, are described and illustrated. *Inonotus multisetifer* is proposed as a new species characterized by resupinate basidiomata with round to angular pores, 6–9 per mm, acute setal hyphae embedded in trama, subulate hymenial setae, and globose to subglobose basidiospores. *Trametes cingulata* constitutes a first record from Brazil.

KEY WORDS — mycodiversity, neotropics, *Polyporales*, *Hymenochaetales*

Introduction

The Atlantic Rain Forest, which has 20,000 species of plants of which 6000 are endemic, holds today less than 8% of its original extent in Brazil and has been rated as one the world's top five biological hotspots (Mittermeier et al. 1999, SOS Mata Atlântica/INPE 2009).

The state of São Paulo is located in southeastern Brazil and has an area of 248,808.8 km². Mostly inserted in the Atlantic Forest domain (68%), the state contains remnants of rain forest, *Araucaria* forest, seasonal semideciduous forest, transitions between them, and remnants of vegetation of restinga and mangrove (Kronka 2005, SOS Mata Atlântica/INPE 2009).

Currently, the natural vegetation cover of the state amounts to only 13.94% of its surface (Kronka 2005, Nalon et al. 2008), concentrated mainly along the coastal zone, where there is a concentration of natural areas of Integral Protection (Xavier et al. 2008). In the northwest region, only minor conservation units are found (Kronka 2005, Xavier et al. 2008), with the majority of the remaining forest located in private areas where their conservation is key to biodiversity conservation in the State.

The mycodiversity in northwest São Paulo State is almost unknown; currently, only 36 species of basidiomycetes have been cited from this region (Xavier-Santos 2003, Abrahão et al. 2008, 2009).

As part of a wider project dealing with biodiversity of remnant forest fragments of the northwest region of São Paulo State, this study reports a new species of *Inonotus* and a new record of *Trametes cingulata* from Brazil, including descriptions, illustrations, and discussions on the species.

Materials & methods

Collections were performed between March 2007 and December 2008 in Semidecidual Stacional Forest fragments in northwest São Paulo State, Brazil.

The material was studied following the classical methods for polypores (Gilbertson & Ryvarden 1986, Ryvarden 1991, 2004). Micromorphological observations were made from material mounted in 5% KOH and Melzer's reagent; measures were made in 5% KOH in a Leica DM 1000 microscope.

Vouchers are deposited in SP, SJRP, and O (acronyms follow Holmgren & Holmgren 1998).

Nomenclature and classification systems follow those of Kirk et al. (2008), Centraalbureau voor Schimmelcultures (www.cbs.knaw.nl), and Index Fungorum (www.indexfungorum.org).

Taxonomy

Inonotus multisetifer Abrahão & Gugliotta, sp. nov.

FIGS 1–4

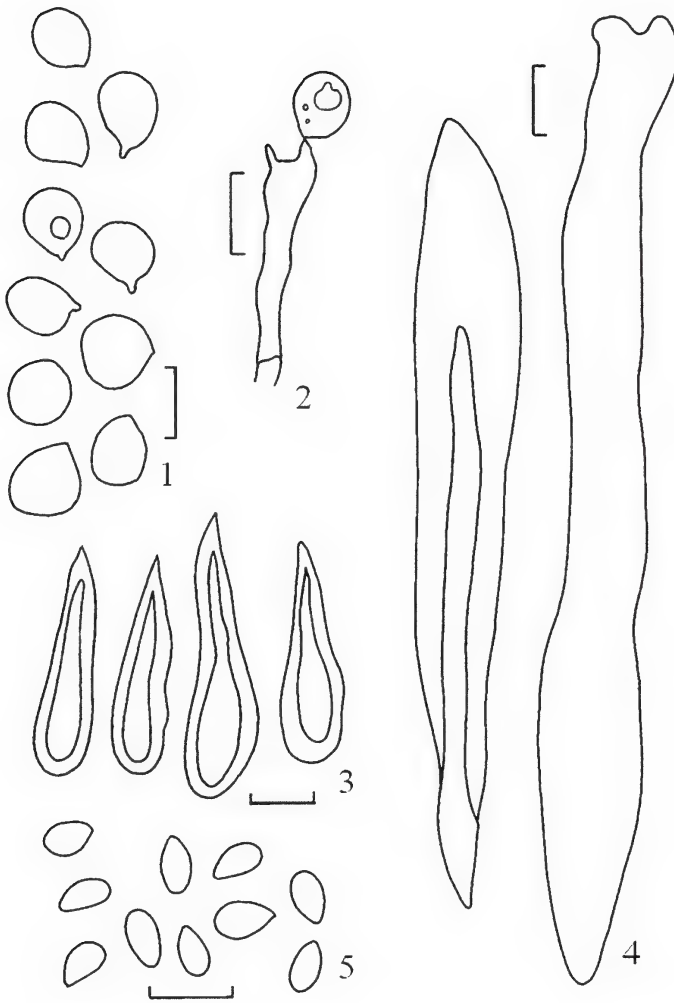
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Differs from *Inonotus adnatus* in larger basidiomata, slightly narrower setal hyphae, smaller scant hymenial setae, and larger ($8.8\text{--}11.2 \times 8.8\text{--}10\ \mu\text{m}$) globose to subglobose basidiospores.

TYPE: BRAZIL. São Paulo State: São João de Iracema, São Francisco Farm, G5 area ($20^{\circ}28'25''\text{S } 50^{\circ}17'36''\text{W}$), 17.IV.2007, M.C. Abrahão 270 (holotype, SP393791; isotype, SJRP).

ETYMOLOGY: Referring to the abundant setal hyphae.

BASIDIOMA annual, resupinate, up to 8 cm in diameter, ca. 4 mm thick, strongly adnate, hard and dense when dry. MARGIN thin to absent. PORE SURFACE ochraceous to brown, pores round to angular, 6–9 per mm, invisible to the naked eye; tubes dark brown, up to 4 mm deep. CONTEXT homogeneous, very thin, virtually absent in parts, cinnamon. HYPHAL SYSTEM monomitic, IKI–; generative hyphae simple-septate, thin to thick-walled, golden to rusty brown, $(2.5\text{--})3.75\ \mu\text{m}$ wide. SETAL HYPHAE abundantly present, dark brown, acute, thick-walled, $100\text{--}200 \times 8.75\text{--}16.25\ \mu\text{m}$, embedded in trama and not projecting. HYMENIAL SETAE scant and in some sections totally absent, apparently found only in the young pores and in the pores mouths, dark brown, subulate, thick-walled, $33.75\text{--}45 \times 8.75\text{--}10\ \mu\text{m}$. BASIDIA clavate, hyaline, with four sterigmata,



FIGURES 1–5 (scale bars = 10 μm). FIGS. 1–4: *Inonotus multisetifer* (SP393791, holotype) — 1, basidiospores; 2, basidia and immature basidiospore; 3, hymenial setae; 4, setal hyphae. FIG. 5. *Trametes cingulata* (SP416154) — basidiospores.

22.5–23.75 $\times$ 5–6.25 μm . BASIDIOSPORES globose to subglobose, IKI–, slightly thick-walled, hyaline to pale yellow, 8.75–11.25 $\times$ 8.75–10 μm .

SUBSTRATE & DISTRIBUTION —dead hardwood; known only from the type locality in Brazil.

ADDITIONAL SPECIMENS EXAMINED —*Inonotus adnatus*: COSTA RICA. PUNTARENAS: La Amistad Pacifico, Estacion Progreso, near the station, alt 1180 m, 27.IX.2000, L. Ryvardeen 42795 (O316222-079, isotype). *Inonotus micantissimus*: BRAZIL. RIO GRANDE DO SUL STATE: São Salvador, III.1944, S.J. Rick 20691 (PACA8878, holotype). *Inonotus pegleri*: TANZANIA. EASTERN PROVINCE: KILOSA DISTRICT, Mikumi National Park, on dead fallen tree, alt 487 m, 30.IV.1968, D.N. Pegler T725 (K167869, holotype; O296, isotype).

COMMENTS — *Inonotus multisetifer* is characterized by resupinate basidiomata, presence of acute setal hyphae in the trama, scant subulate hymenial setae, and globose to subglobose slightly thick-walled hyaline to pale yellow basidiospores. This combination of characters relate this species to other *Inonotus* resupinate species that also have setal hyphae, hymenial setae, and basidiospores varying from globose, subglobose to ellipsoid: *I. adnatus* Ryvardeen, *I. micantissimus* (Rick) Rajchenb., and *I. pegleri* Ryvardeen. The main diagnostic characteristics are shown in TABLE 1.

TABLE 1. Key features of *Inonotus multisetifer* and morphologically related species.

	<i>I. multisetifer</i>	<i>I. adnatus</i>	<i>I. micantissimus</i>	<i>I. pegleri</i>
Basidioma (thickness)	≤ 8 cm diam. (~4 mm)	≤ 4 cm diam. (~3 mm)	10 × 4 cm (≤14 mm)	12 cm (≤ 15 mm)
Pores/mm	6–9	6–9(–10)	5–6	4–6
Generative hyphae: walls; color	Thin to thick; golden to rusty brown	Thin to thick; golden to rusty brown	Slightly thick; yellowish to chestnut	Thin to thick; hyaline to light-brown
Hymenial setae (µm)	33.8–45 × 8.8–10	26.2–56.2 × 10–12.5	22.5–27.5 × 6.2–11.2	20–21.2 × 6.2–8.8
Setal hyphae (µm)	100–200 × 8.8–16.2	≤ 150 × 8.8–17.5	160–300(–400) × 8.8–20*	125–520 × 7.5–21.2
Basidiospore shape; color	(Sub)globose; hyaline to yellowish	Globose; yellowish	(Sub)globose, (apiculate); hyaline to pale melleous	Globose; hyaline to yellowish
Basidiospore size (µm)	8.8–11.2 × 8.8–10	7–8.8 diam.	11.25 × 8.8–10	6–7 µm wide

*(width up to 25 µm in KOH)

Inonotus multisetifer is morphologically most closely related to *I. adnatus*, a species known only from the type locality in Costa Rica (Ryvardeen 2002, 2004, 2005). *Inonotus adnatus* presents a smaller basidioma, slightly wider setal hyphae, larger and ventricose hymenial setae, and smaller globose basidiospores. Hymenial setae are abundant in the type specimen of *I. adnatus* but scant to mostly absent in *I. multisetifer*.

Inonotus micantissimus, which also occurs in Brazil (Rajchenberg 1987, Baltazar et al. 2010), has larger pores, longer setal hyphae, smaller hymenial setae, and basidiospores with abundant oily contents.

Inonotus pegleri is easily separated by the combination of larger pores, smaller hymenial setae, longer setal hyphae, smaller basidiospores, and its limited distribution in Africa (Ryvarden 2005).

Trametes cingulata Berk., Hooker's J. Bot. Kew Gard. Misc. 6: 164, 1854 FIG. 5

BASIDIOMA annual, lignicolous, sessile pileate to pseudostipitate, dimidiate, many basidiomata from one stipe or solitary; pileus semicircular, applanate and thinner near the margin, with contracted base, soft when fresh and flexible when dry, $0.7\text{--}4.6 \times 0.7\text{--}3.9 \times 0.1\text{--}0.8$ cm. PILEAR SURFACE in brown and cream tints, concentrically zonate, glabrous. MARGIN round, concolorous with the pilear surface, entire, glabrous and sterile. Stipe when present circular, cream to brown, glabrous, dense, $0.2\text{--}1.1 \times 0.2\text{--}0.9 \times 0.5\text{--}1.3$ cm. PORE SURFACE poroid, lighter than pilear surface, pores angular to elongated, 7–9 per mm; tubes stratified, forming up to 4 distinct layers, light-brown, up to 2 mm deep; dissepiments thin to slightly thick, lacerate. CONTEXT duplex, the upper layer fibrous, greyish to blackish, thin, at least at the base separated by a thin black line from the lower part, the latter light-brown, corky, up to 6 mm thick at the base, negative reaction in KOH. HYPHAL SYSTEM trimitic, IKI–; generative hyphae hyaline, with clamps, thick-walled, $2.5\text{--}3.75$ μm wide; skeletal hyphae yellowish, thick-walled, abundant in the basidioma, golden and solid in the context, $3.75\text{--}6.25$ μm wide; binding hyphae hyaline to yellow, thick-walled to semisolid in the context, slightly irregular and moderately branched, frequent, $1.25\text{--}3.75$ μm wide. CYSTIDIA absent. BASIDIA clavate, hyaline, thin-walled, $11.25\text{--}12.5 \times 3.75\text{--}5$ μm . BASIDIOSPORES obovoid, hyaline, IKI–, smooth, thin-walled, $5\text{--}6.25 \times (2.5\text{--})3.75$ μm .

SUBSTRATE & DISTRIBUTION — causing white-rot in twigs of dead angiosperm. Previously cited from Asia, Africa, and Australia (Ryvarden & Johansen 1980, Young et al. 2004, Gilbert et al. 2008) and Costa Rica and Venezuela in America (Ryvarden & Iturriaga 2001, Mata et al. 2007).

MATERIAL EXAMINED — BRAZIL. SÃO PAULO STATE: Palestina, Boa Vista Farm, P6 area ($20^{\circ}19'16''\text{S } 49^{\circ}30'17''\text{W}$), 10.XII.2008, A.M. Gugliotta 1410 (SP416154, SJRP); São João de Iracema, São Francisco Farm, G5 area ($20^{\circ}28'25''\text{S } 50^{\circ}17'36''\text{W}$), 27.XI.2007, M.C. Abrahão 319 (SP393683). INDIA. BEHAR: Soane River [BIHAR: Son River], on dead wood, J. Hooker s.n. (K167868, isotype).

COMMENTS — Macroscopically the species may be recognized by its glabrous basidioma in brown and cream tints (Ryvarden & Johansen 1980). The angular to elongated pores, duplex context with a greyish to blackish fibrous upper

layer, separated by a thin black line from the lower light-brown corky part, and obovoid, hyaline, thin-walled basidiospores support the identification. It constitutes a new record from Brazil.

Acknowledgments

The authors acknowledge Dr. Leif Ryvarden for identification of *Trametes cingulata* (SP393683). We are grateful to FAPESP (first author's fellowship process nº 2006/58786-6 and BIOTA thematic project process nº 2004/04820-3) for financial support. We also extend our thanks to Gerardo Lucio Robledo and Rosa Mara Borges da Silveira, who kindly reviewed the manuscript.

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***Manoharachariella indica* sp. nov. from the Western Ghats, India**KUNHIRAMAN C. RAJESHKUMAR¹ & SANJAY K. SINGH²

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ABSTRACT — *Manoharachariella indica* is described as a new species from India, differing from *M. lignicola*, the only other described species of the genus, by larger conidia, longer conidiophores, and additional conidiophore septa.

KEY WORDS — anamorphic fungi, dematiaceous hyphomycete, Tamhini Ghats, Maharashtra

Introduction

The Western Ghats, one of the world's most diverse 'biodiversity hot spots', is unique and diverse for its fungal wealth. Studies on microfungi and exploration of fungal diversity from different microhabitats in the Western Ghats are routinely conducted by the National Fungal Culture Collection of India (NFCCI) (Rajeshkumar et al. 2010, 2011a, b; Singh et al. 2009, 2010). During a September 2011 expedition to explore microfungal diversity in the Mulshi and adjoining areas we discovered a dematiaceous hyphomycete on a dead twig from the bamboo dominating the evergreen patch in the valley of the Tamhini Ghats.

Bagyanarayana et al. (2009) recently established *Manoharachariella* for *M. lignicola* Bagyan. et al. from litter in Andhra Pradesh, India. Monoblastic integrated conidiogenous cells that produce solitary doliiform obpyriform dictyoseptate apiculate conidia characterize this dematiaceous hyphomycete genus. We describe a second species in this genus.

Materials and methods

ISOLATES AND MORPHOLOGY — Conidia were isolated directly from the surface of a dead twig and observed under a Nikon binocular stereomicroscope (Model SMZ-1500 with Digi-CAM, Japan). Single conidial cultures were established on 2% potato dextrose agar plates (PDA; Crous et al. 2009). For morphotaxonomic studies and photomicrographs, Zeiss (AXIO Imager 2, Germany) and Olympus (Model CX-41,

Japan) microscopes were used. Conidia and conidiophores were mounted in lactic acid cotton blue and measured using an ocular micrometer (and confirmed with software available with the Zeiss microscope), with 30 observations per structure. Culture colony characteristics were studied on two different media: 2% malt extract agar (MEA) and PDA (Crous et al. 2009). Herbarium specimens are deposited in the Ajrekar Mycological Herbarium (AMH), and the culture NFCCI 2630 was accessioned and preserved at NFCCI; WDCM-932, Agharkar Research Institute, Pune, India.

Taxonomy

Manoharachariella indica Rajeshkumar & S.K. Singh, sp. nov.

PLATES 1–3

MYCOBANK MB 563453

Differs from *Manoharachariella lignicola* by larger conidia, longer conidiophores, and additional conidiophore septa.

TYPE: India, Maharashtra, Tamhini Ghats, on dead twigs, September 2011, K.C. Rajeshkumar (**Holotype**, AMH 9438; ex-type culture NFCCI 2630).

ETYMOLOGY: *indica*, referring to the country where this fungus is native.

Colonies effuse, thin, mid to dark brown or blackish brown, mycelium superficial or semi immersed or immersed, hyphae pale to dark brown, smooth, 4–6 μm . Stroma none. Setae and hypopodia absent. Conidiophores macronematous, mononematous, straight or flexuous, sparingly branched, arising laterally and apically from the immersed mycelium, 5–15-septate, smooth, pale to dark brown 76–276 $\times$ 6–10.8 μm . Conidiogenous cells monoblastic, integrated, terminal or

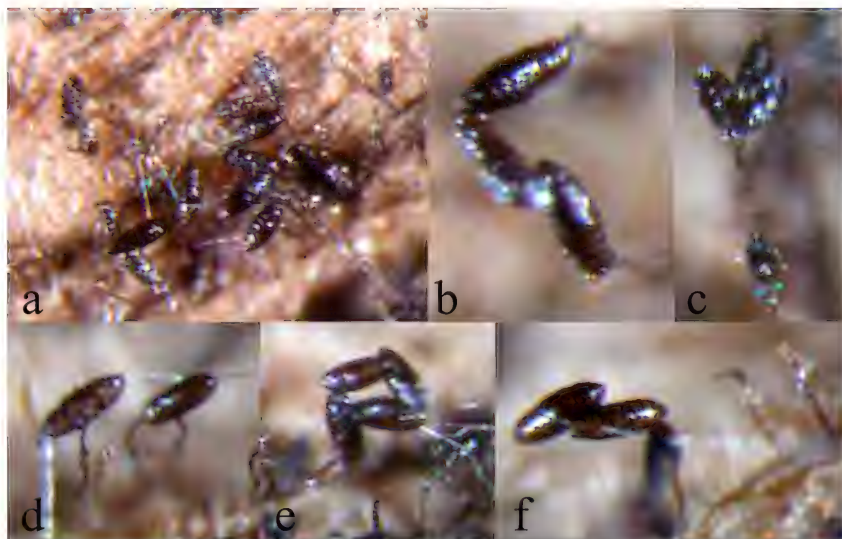


FIG. 1. *Manoharachariella indica* (holotype): a. Habit. b. Sticky conidia in false chain. c. Terminal and lateral conidiogenesis. d–f. Conidiophores and conidia in nature.

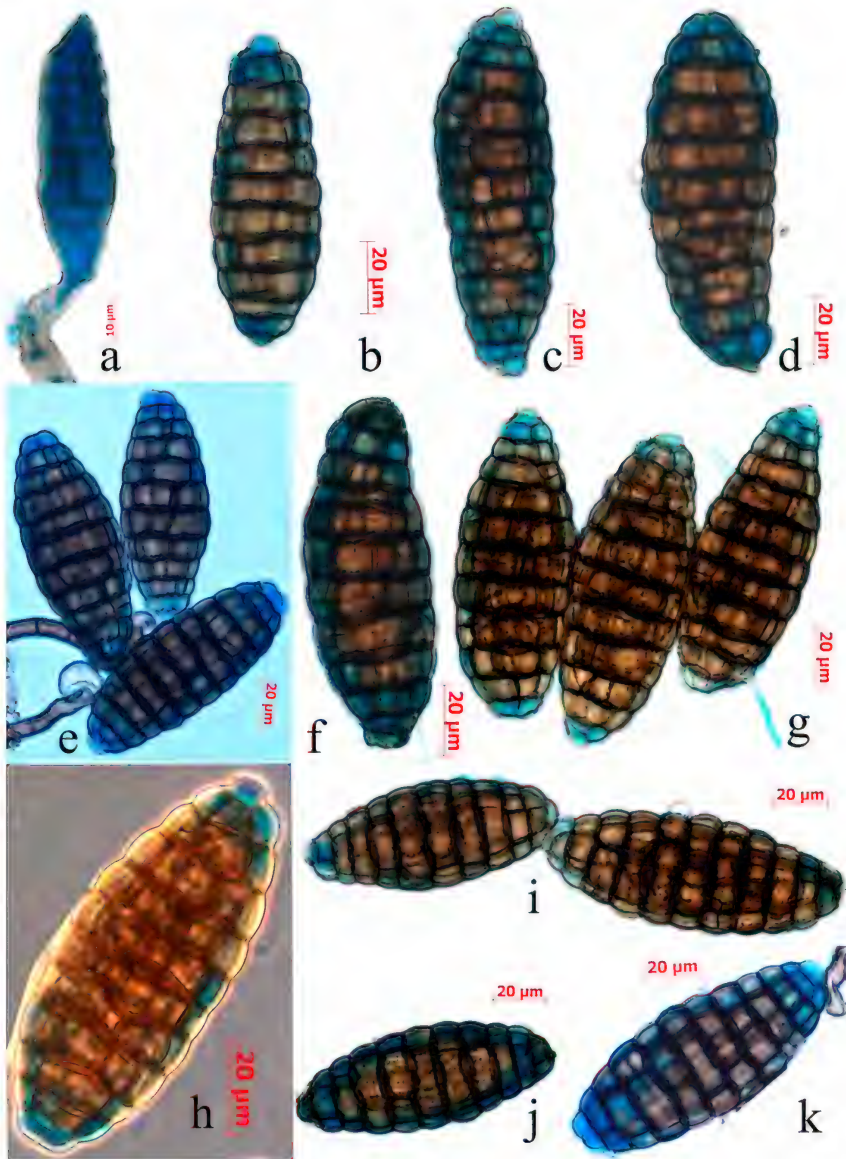


FIG. 2. *Manoharachariella indica* (holotype): a. Conidial development. b–c. Apiculate conidia. d, f–g, j–k. Variation in conidial size and shape. e. Conidia with conidiophores. h. Conidia under phase contrast view. i. Sticky conidia in false chain.

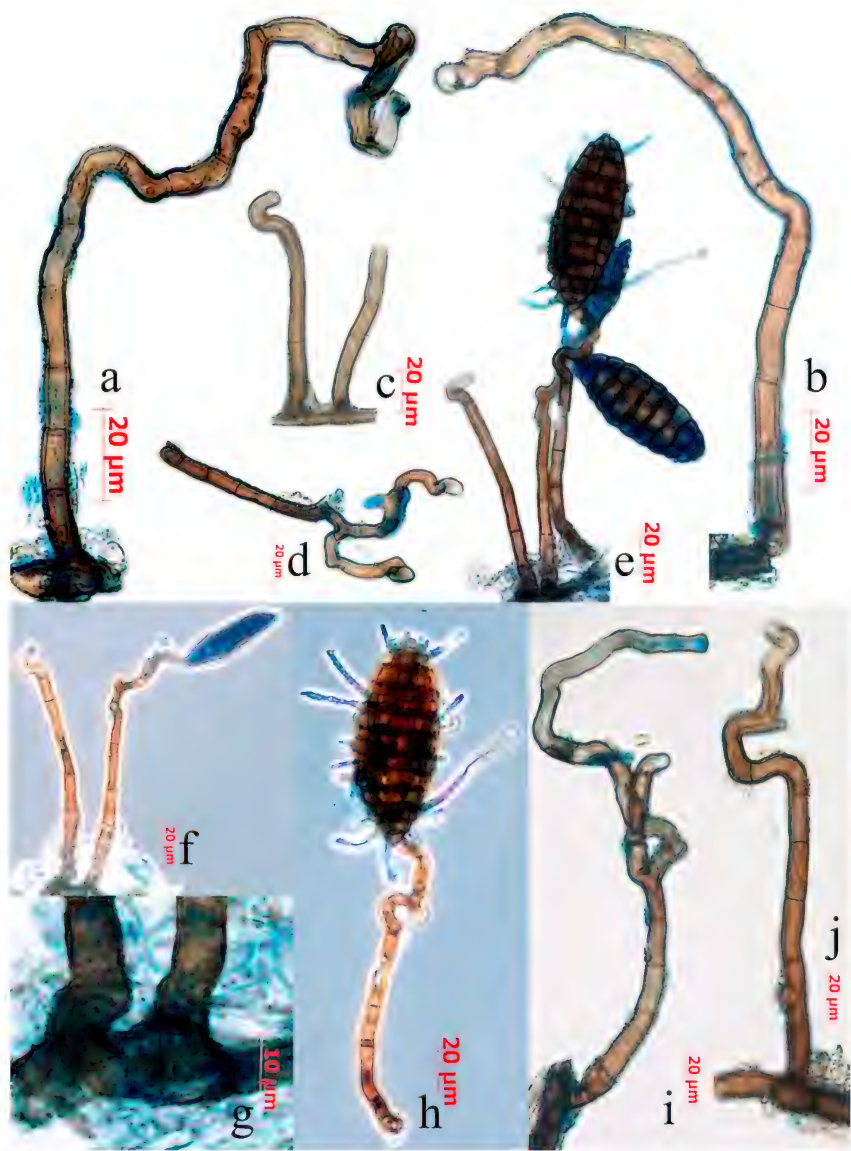


FIG. 3. *Manoharachariella indica* (holotype): a–b. Mature conidiophores. c. Young conidiophores. d. Hyphal branching and initiation of conidiophores in nature. e. Conidiophores and attached conidia. f. Conidiophores with young conidia. g. Foot cells of conidiophores. h. Mature conidia attached with conidiophores, showing multi-polar germination. i. Branched conidiophore. j. Simple conidiophore.

intercalary, pale to mid brown, $4.4\text{--}8.6 \times 4\text{--}7.6\ \mu\text{m}$. Conidia solitary, dry or sticky, acropleurogenous, simple, doliiform, obpyriform, ellipsoidal or irregular, apiculate, apiculus rounded or obtuse, smooth, dictyoseptate, longitudinally 10–13-septate, tiered, pale to dark brown or blackish brown, apical and basal tiers hyaline or subhyaline. $84\text{--}110(\text{--}120) \times 31\text{--}44\ \mu\text{m}$; young conidia hyaline or subhyaline, $56 \times 17\ \mu\text{m}$.

Conidia in nature are dry and/or sticky and also form false chains of 2–3 conidia. Conidial germination was observed as being multi-polar in moist chambers, but also after 12 hours of inoculation on PDA plates.

Colonies on PDA and MEA very slow growing, 1–2 mm diam after 14 days, pale brown, velutinous; reverse pale brown.

TELEOMORPH: not observed.

Discussion

The monotypic *Manoharachariella* with its type species *M. lignicola* somewhat resembles *Septosporium* Corda (especially *S. rostratum* M.B. Ellis, 1961) in having dictyoseptate beaked conidia but differs in the absence of setae, unbranched conidiophores, and percurrent conidiogenous cells. Two species of *Xenosporium* Penz. & Sacc. — *X. africanum* Piroz. (Deighton & Pirozynski 1966) and *X. boivinii* S. Hughes (Hughes 1978)— resemble *Manoharachariella* in conidial shape but differ in their monoblastic, acrogenous or acropleurogenous, obpyriform, apiculate, smooth and dictyoseptate conidia that are transversely longitudinally septate. In addition, secondary conidia are absent in *Manoharachariella* but present in *Xenosporium*. *Manoharachariella*, like *Bioconiosporium* Bat. & J.L. Bezerra (Ellis 1976), produces solitary dictyoseptate conidia but the conidia are monoblastic with single apiculus and lack the two large protuberances that are prominent in *Bioconiosporium*. Conidia in both *Monodictys* S. Hughes (Ellis 1971) and *Manoharachariella* are monoblastic, dictyoseptate, and acrogenous but those in *Manoharachariella* are apiculate and tiered. Bagyanarayana et al. (2009) noted all these differences when they established *Manoharachariella*.

Manoharachariella lignicola is differentiated from *M. indica* by its smaller ($42.5\text{--}50.5 \times 25\text{--}32\ \mu\text{m}$) 7–9 transversely septate conidia and smaller conidiophores ($\leq 35\ \mu\text{m} \times 3\text{--}4.5\ \mu\text{m}$) with fewer (1–3) septa.

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***Russula jilinensis* sp. nov. (Russulaceae) from northeast China**GUO-JIE LI^{1,2}, SAI-FEI LI¹, XING-ZHONG LIU¹ & HUA-AN WEN^{1*}¹State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences,
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ABSTRACT — *Russula jilinensis* (subg. *Coccinula* sect. *Laetinae*), is described from Changbai Mountains, northeast China. The new species is distinguished by its bright red glabrous pileus with a cinnamon tinged disc, slightly yellowish context, dark yellow to ocher spore print, and pileipellis with septate pileocystidia. The morphological characteristics are illustrated in detail and compared with those of similar species. Identification of *R. jilinensis* was supported by the molecular phylogenetic analysis based on the ribosomal DNA internal transcribed spacer regions (ITS).

KEY WORDS — *Russulales*, taxonomy, morphology, *Basidiomycota*

Introduction

Northeast China, including Liaoning, Jilin, and Heilongjiang Provinces and the eastern part of Inner Mongolia Autonomous Region, covers an area of 1.236×10^6 km² (39°–53°30' N 115°–135°E) within the temperate to boreal continental climate zones. Plant communities range from grassland (eastern Inner Mongolia) to broadleaf forest (southern Liaoning), while the three main mountain systems (Great Hinggan, Lesser Hinggan, Changbai) are mostly covered by coniferous or mixed coniferous–broadleaf forests. The main trees in northeast China are *Pinus pumila*, *P. koraiensis*, *Larix gmelinii*, *Betula platyphylla*, *Abies nephrolepis*, *Picea jezoensis*, and *Quercus mongolica* (Jiang et al. 2003, Xu et al. 2008). In summer and early autumn, these ectomycorrhizal commensal plants facilitate the fruiting of ectomycorrhizal basidiomycetes, among which the members of *Russula* Pers. are very common.

Russula is widely distributed from western Europe to North America in the northern hemisphere (Romagnesi 1967, Singer 1986, Sarnari 1998, Miller & Buyck 2002, Bau et al. 2008). Although some new species and varieties have been reported from southern and southwestern China (Singer 1935, Chiu 1945, Ying 1983, 1989, Bi & Li 1986, Wen & Ying 2001, Wang et al. 2009), *Russula*

species have not been studied systematically elsewhere in China, including the north. Because of its taxonomic difficulty and the insufficiency of intensive taxonomic studies in China (Wang et al. 2009), many *Russula* species reported from northeastern China are identified using European or American names and lack voucher support (e.g., Teng 1996, Xie et al. 1986, Li & Bau 2003, Bau 2004). As now in North America (Adamcik & Buyck 2010, 2011; Buyck & Hofstetter 2011), the lack of endemic systematic studies has probably led to incorrect estimates of the extent of *Russula* diversity in northeastern China,.

Although many wood-inhabiting fungi were recently reported from northeastern China (Dai & Penttilä 2006, Yuan et al. 2006, Wei & Dai 2007, Dai et al. 2008, Xiong et al. 2008, Dai 2010), only a few *Russula* species have been reported from this area (Song et al. 2007). In addition, many virgin and natural forests in this area have not yet been intensively investigated. During a survey on *Russulales* in the Changbai Mountains, one interesting *Russula* was found, which is described here as a new species.

Materials & methods

Specimens were collected from the Changbai Mountains, Antu County of Jilin Province, from 2008 to 2010. Macromorphological characteristics of the fresh fruiting bodies were recorded in field notes. Color names and codes follow Ridgway (1912) and spore print colors were categorized according to Romagnesi (1967). Collections were oven-dried at 50–60 °C until their water content was <15% prior to microscopic examination. Solutions of 10% FeSO₄ and sulfovanillin (SV) were used for testing the chemical reaction of dried specimens. Specimens for microscopic examinations were hand-sectioned and rehydrated in a 5% KOH solution prior to observation under a Nikon Eclipse 80i microscope. Basidiospores (examined in Melzer's reagent), basidia, pleurocystidia, cheilocystidia and elements of the pileipellis and stipeipellis were measured, with at least 20 elements measured for each character. The abbreviation (n/m/p) = n spores from m basidiospores of p specimens. Basidiospore dimensions are shown as (a–) b–c (–d), with the range b–c including 90% of the measured values and the extreme values shown in parentheses. The spore quotient (Q) = spore length divided by spore width. Q (in bold) = average quotient value + standard deviation. Spore ornamentations and apiculi were not included in measurements. Further explanations of basidiospore data follow Yang (2000). Scanning electron images were also captured with an FEI Quanta 200 electron microscope. Abbreviations of herbarium names follow Holmgren et al. (1990). Specimens cited were deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS).

For phylogenetic studies, genomic DNA was extracted from dried fruiting bodies briefly crushed with quartz sand in liquid nitrogen. DNA template for amplification was prepared using a BioTeke plant DNA rapid extraction kit. PCR was conducted with an Eastwin Dragon Black Vajra thermo cycler using ITS1 (5'-GTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCGCTTATGATATGC-3') primers (Shanghai Sangon, Beijing TransGen Biotech), EasyTaq DNA polymerase, dNTPs, and PCR buffer to amplify the ITS1+5.8S+ITS2 region. Amplification of a 50 µL mixture — 35.5 µL ddH₂O, 5 µL

PCR buffer, 4 µl dNTP (2.5 mmol/L), 1 µl DNA template, 2 µl (10 µmol/L) each of two primers, 0.5 µl (5u/µl) Taq DNA polymerase — followed a protocol of 5 min at 94°C (initial denaturation), 35 cycles of 1-min at 94°C (denaturation) + 1 min at 56°C (annealing) + 1 min at 72°C (extension), and a final 10-min extension at 72°C. The PCR products were electrophoresed in ethidium bromide-stained agarose gel and sequenced by SinoGenoMax Co. Ltd, using an ABI 3730XL Analyzer and ABI BigDye 3.1 Cycle Sequencing Kit. The sequences were deposited in GenBank.

Sequences were aligned and edited in ClustalX (Thompson et al. 1997) and Bioedit (Hall 1999). Phylogenetic analysis was performed with MEGA version 4.1 Beta (Tamura et al. 2007), and Minimum Parsimony trees were constructed with the test of inferred phylogeny a bootstrap of 1000 replications and a random seed (Eck & Dayhoff 1966, Rzhetsky & Nei 1992). The MP tree was obtained using the Close-Neighbor-Interchange algorithm with search level in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree was drawn to scale, with branch lengths calculated using the average pathway method and in the units of the number of changes over the whole sequence (Nei & Kumar 2000). All positions containing gaps and missing data were eliminated from the data set (Complete Deletion option). The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. Of the 424 positions in the final dataset, 253 are parsimony informative. The ITS1-5.8S-ITS2 sequences of *Albatrellus ovinus* (Schaeff.) Kotl. & Pouzar and *Bondarzewia mesenterica* (Schaeff.) Kreisel (as *B. montana*) were used as outgroups (Wang et al. 2009).

Taxonomy

Russula jilinensis G.J. Li & H.A. Wen, sp. nov.

FIGS 1–4

MYCOBANK MB 563682

Differs from *Russula integra* by shorter basidia and pleurocystidia and smaller spores.

ETYMOLOGY: named after the type locality.

TYPE: CHINA, Jilin Province, Antu County, Erdaobaihe, Changbai Mountains, 42°24'N 128°04'E, alt. 761 m, on ground in mixed coniferous–broadleaf forest dominated by *Pinus koraiensis* and *Picea*, 1 August 2008, M.X. Zhou & Y. Gafforov 08004 (Holotype, HMAS194253, GenBank GU966632).

PILEUS 6.1–7.3 cm broad, first hemispheric, convex, then more or less convex to plano-convex, depressed weakly in the center when mature, colors Jasper Red to Coral Red (xiii3'–5') when juvenile, Pompeian Red to Dragon's-blood Red (xiii3'i–5'i) with age, Nopal Red to Brazil Red (13i–5i) when dried; center Vinaceous-Cinnamon to Pinkish Cinnamon (xxix13''b—15''b) when juvenile, Orange-Cinnamon to Cinnamon (xxix13''–15'') with age, Mikado Brown (xxix13''i) when dried, Nopal Red (13i) in SV, color unchanged in FeSO₄; viscid when wet, glabrous, smooth, non-pruinose when dried, pellis peeling 1/2 to the disc; margin obtuse, not or very slightly striate when old, never cracked. CONTEXT 1.0–1.5 cm thick from stipe top to pileus center, brittle, white (LIII), Naples Yellow (xvi19'd) when old or dried, color unchanged when bruised;



FIG.1: *Russula jilinensis* (Holotype HMAS 194253).



FIG.2: *Russula jilinensis* (HMAS 262395). Scale bar = 1 cm.

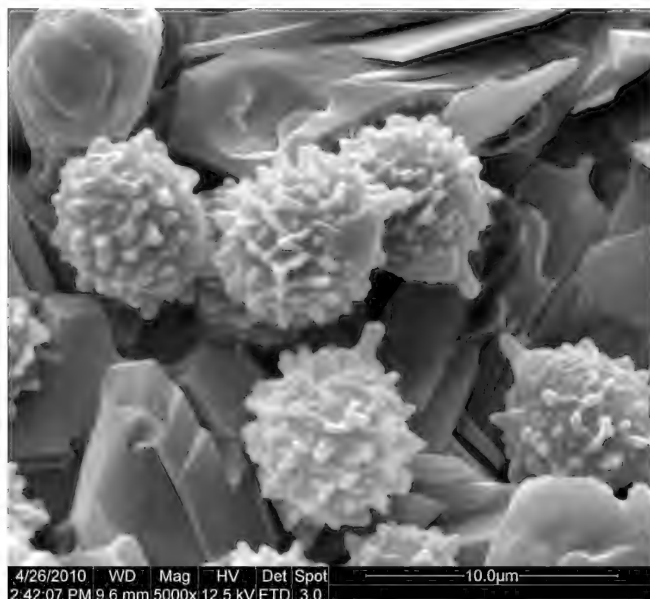


FIG. 3: *Russula jilinensis* (Holotype HMAS194253).

Scanning electron microscope (SEM) photo. Basidiospores. Scale bar = 10 μm.

taste mild; odor none or indistinct. LAMELLAE 4–7 mm broad, 9–12/cm at the edge, no lamellulae, decurrent to more or less adnate, not forked, fragile, Pale Yellow-Orange (III15f) first, Pale Orange-Yellow (III17f) when mature or dry, color unchanged in FeSO_4 . STIPE clavate, enlarged towards the base, 9.2×1.8 – 2.1 cm, smooth, solid but irregularly hollowing when old, white (LIII) when juvenile, becoming Baryta Yellow, Wax Yellow, or Primuline Yellow (IV21f, XVI21', XVI19', respectively) towards the base when old or dry, Brazil Red (I5i) in SV, no color change in FeSO_4 . SPORE PRINT dark yellow, ocher, IIC.

BASIDIOSPORES (80/3/3) $(6\text{--}7\text{--}8\text{--}8.5) \times (5.5\text{--}6\text{--}7)$ μm, average 7.5×6.3 μm, [Q= (1.02) 1.06–1.28 (1.31), Q= 1.18 ± 0.06], subglobose to ellipsoid, without oil droplet, plage indistinct and amyloid; ornamentation amyloid, mostly isolated, rarely linked by fine lines and not forming a mesh, warts conic to cylindrical, ≤ 0.4 – 1.0 μm tall. BASIDIA (33–)38–44 $\times$ 11–13(–14) μm, clavate to subclavate, broadly tapered towards the base, 4-spored, sometimes 2-spored, hyaline in KOH, occasionally containing a large droplet; mature basidia projecting ≤ 15 – 10 μm above the subhymenium; sterigmata 3–5 μm. PLEUROCYSTIDIA (50–)53–56(–63) $\times$ (8–)9–10(–11) μm, rare, emergent, fusiform, clavate to subclavate, often with a subacute tip, sometimes with a frayed small appendage and dense crystal inclusions, projecting ≤ 35 μm above the subhymenium, color unchanged in FeSO_4 , dark grey in SV reaction dark grey.

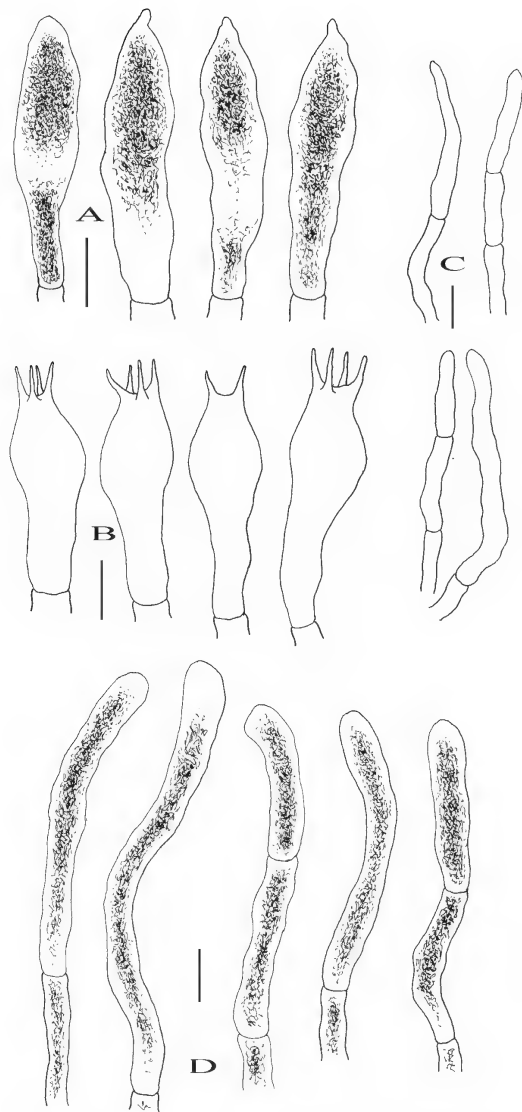


FIG. 4: *Russula jilinensis* (Holotype HMAS 194253).
A. Pleurocystidia; B. basidia; C. terminal elements of pileipellis; D. pileocystidia.
Scale bars = 10 μm .

CHEILOCYSTIDIA mostly similar to pleurocystidia. PILEIPELLIS two-layered, 100–125 μm thick, gelatinous; epicutis an ixotrichoderm, epicuticular hyphae 3–6 μm broad, septate, mostly unbranched, erect to suberect, tangled, hyaline

in KOH, terminal cells thin-walled, cylindrical, with obtuse, undifferentiated, often tapered ends; pileocystidia $48\text{--}64 \times 5\text{--}7\text{ }\mu\text{m}$, dispersed, numerous in surface, scattered, clavate, subclavate to cylindrical, with 0–2 septa, contents crystalline, granulate, slightly turning grey in SV; subcutis hyphae recumbent, filamentous, interwoven, branched, septate, $2.5\text{--}5\text{ }\mu\text{m}$ diam., hyaline to pale yellowish brown in KOH; trama composed of subspherical to angular $13\text{--}24\text{ }\mu\text{m}$ diam sphaerocysts and connective hyphae. STIPITIPPELLIS composed mostly of interwoven branched elongated $3\text{--}6\text{ }\mu\text{m}$ diam hyaline hyphae with inflated cells; caulocystidia absent. Clamps or laticiferous hyphae absent in all tissues.

ECOLOGY: Solitary on soil in mixed coniferous–broadleaf forests, July–August. Known only from the Changbai Mountains. Edibility: unknown.

ADDITIONAL SPECIMENS EXAMINED: CHINA, JILIN PROVINCE, ANTU COUNTY, Heping Forest Farm, $43^{\circ}07'N$ $128^{\circ}54'E$, alt. 1014 m, in coniferous forest dominated by *Pinus*, 22 July 2010, L.D. Guo, X. Sun, G.J. Li & L.J. Xie 20100054 (HMAS262395, GenBank HQ693525); Changbai Mountains Forest Ecological System Research Center, $43^{\circ}23'N$ $128^{\circ}05'E$, alt. 811 m, in mixed coniferous–broadleaf forest, 25 July 2010, X. Sun & G.J. Li 20100410 (HMAS262364, GenBank HQ693524).

Discussion

Russula jilinensis is macroscopically characterized by its bright red pileus, light-yellow lamellae, and a white stipe without red or purple tinge. It shows superficial affinities with subg. *Coccinula* sect. *Laetinae* Romagn., but its relatively shorter basidia and larger cystidia are more characteristic of subg. *Polychromidia* sect. *Integrinae* Maire (e.g., *R. integra* f. *gigas* Romagn.). The *R. jilinensis* ITS1–5.8S–ITS2 sequences cluster with *R. curtipes* F.H. Møller & Jul. Schäff. (FIG. 5), and a blasting with that of *R. curtipes* (AY061668, Miller & Buyck 2002) through NCBI databank shows a 93% max identification of 97% coverage, confirming that *R. jilinensis* is related to *R. integra/curtipes* group in *Russula* sect. *Integrinae*.

Russula integra (L.) Fr., which is common in Europe, has been reported many times from China and neighboring East Siberia (Teng 1964, Romagnesi 1967, Tai 1979, Xie et al. 1986, Ying et al. 1987, Knudsen & Stordal 1992, Mao & Zhuang 1997, Mao 2000, Bau et al. 2008). However, few specimens of *R. integra* have been recorded from northeast China. *Russula integra* and *R. jilinensis* are similar in spore ornamentation and cystidial shape, and both species are associated with *Pinus* and *Picea* in the subalpine zone (Romagnesi 1967, 1985). However, our microscopic observation followed by intensive comparison of descriptions of *R. integra* by Møller & Schäffer (1935) and Romagnesi (1967) indicates that *R. jilinensis* is a different species. The latter has shorter basidia and pleurocystidia and smaller spores than those of *R. integra* (with spores $8.2\text{--}10 \times 7\text{--}9.2\text{ }\mu\text{m}$, basidia $45\text{--}65 \times 11\text{--}14.5\text{ }\mu\text{m}$, cystidia $82\text{--}120 \times 8.5\text{--}13\text{ }\mu\text{m}$, in Romagnesi 1967).

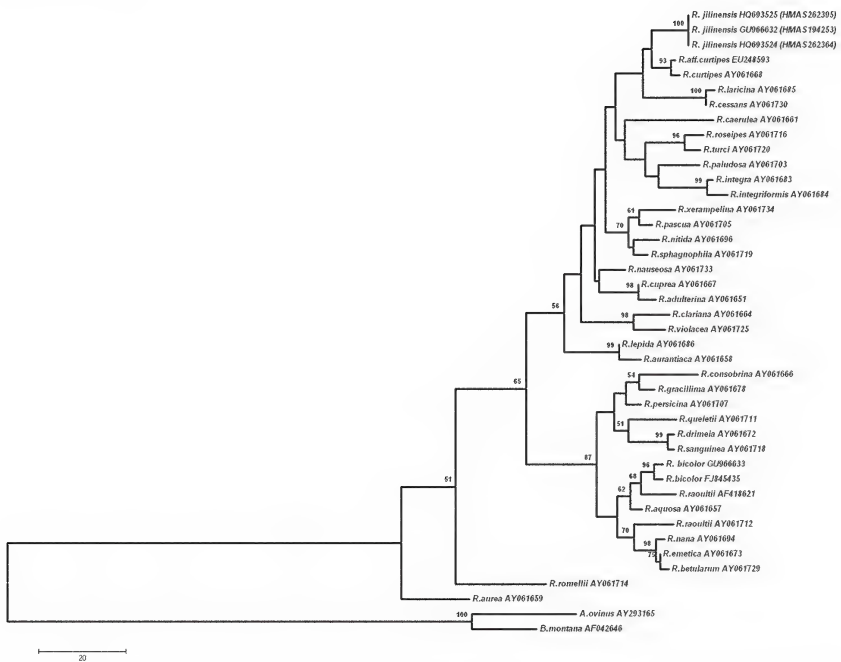


FIG. 5. One of 13 ITS1-5.8S-ITS2-based phylogenies of *Russula jilinensis* and related *Russula* species, using the Maximum Parsimony method. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches.

Another phylogenetically related species is *R. curtipes*, which shares with *R. jilinensis* the short basidia and firm and yellowing context of the *R. integracurtipes* group, but they distinctly differ from each other in pileus color and habitat: *R. curtipes* has a dark wine-red, haematite red, to light russet vinaceous pileus and associates with beech in western Europe with a possible preference for limestone (Romagnesi 1967), while *R. jilinensis* has a brighter red pileus and grows in mixed forests dominated by conifers in efflorescent basalt soils. The reddish pileus of *R. jilinensis* distinguishes it from most of the typical violet- or purple-capped members of *R. sect. Integrinae* such as *R. romellii* Maire and *R. carpini* R. Girard & Heinem. Another species in *R. sect. Integrinae* with a dark-red pileus, *R. rubroalba* (Singer) Romagn., shares a more or less similar pileus color and spore ornamentation but is associated with a deciduous forest and has much longer cystidia than those of *R. jilinensis*.

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***Acrogenospora hainanensis* sp. nov. and new records of microfungi from southern China**

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ABSTRACT — *Acrogenospora hainanensis* is described and illustrated from specimens collected on dead branches in Hainan Province, China. The fungus is characterized by terminal, monoblastic, percurrently extending conidiogenous cells with solitary, spherical or subspherical, non-septate and relatively small conidia. *Acrogenospora gigantospora*, *Nakataea fusispora*, and *Pseudospiropes costaricensis* are newly recorded from China.

KEY WORDS — anamorphic fungi, taxonomy

Introduction

The forests of southern China have a rich mycota, and mycological investigations dealing with many new species have been published recently (Dai & Cui 2006, Cui & Dai 2008, Cui et al. 2009a,b, 2011, Zhang et al. 2009, 2011, Ma et al. 2010). During ongoing mycological surveys in the forests of Hainan and Guangdong provinces, four interesting anamorphic fungi clearly related to *Acrogenospora*, *Nakataea*, and *Pseudospiropes* were collected on dead branches. One of these is an undescribed species of *Acrogenospora*, while the other three species are new records for China.

***Acrogenospora hainanensis* Jian Ma & X.G. Zhang, sp. nov.**

FIG. 1

MYCOBANK MB 564117

Differs from *Acrogenospora verrucispora* in smaller smooth conidia and from *A. sphaerocephala* in smaller conidia and more narrow conidial scars.

TYPE: China, Hainan Province: Tunchang, on dead branches of unidentified broad-leaved tree, 12 April 2011, J. Ma (**Holotype** HSAUP H5509; **isotype** HMAS 146167).

ETYMOLOGY: refers to the province where the type was found.

Colonies on the natural substratum effuse, black, glistening, hairy. Mycelium mostly immersed, composed of branched, septate, subhyaline to pale brown,

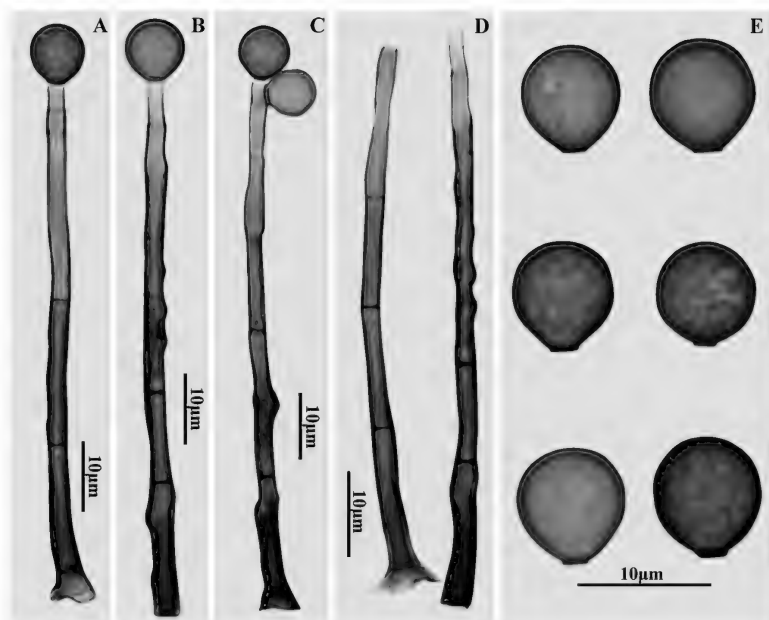


FIG. 1. *Acrogenospora hainanensis* (holotype). A–C. Conidiophores, conidiogenous cells with conidia. Note that in C the mature conidium is pushed sideways following successive percurrent extension of the conidiogenous cells. D. Conidiophores. E. Conidia.

smooth-walled hyphae. Conidiophores macronematous, mononematous, single, unbranched, erect, straight or slightly flexuous, septate, smooth, brown to dark brown, paler towards the apex, 60–80 µm long, 2–3.5 µm thick. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, smooth, pale brown, proliferating percurrently. Conidial secession schizolytic. Conidia solitary, acrogenous, dry, spherical or subspherical, smooth, brown, non-septate, 7.5–9.5 × 7–8.5 µm, with a truncate base 1.5–2 µm wide.

COMMENTS — *Acrogenospora*, with *Monotospora sphaerocephala* Berk. & Broome [= *A. sphaerocephala* (Berk. & Broome) M.B. Ellis] as the type species, is characterized by integrated, terminal, monoblastic, percurrently extending conidiogenous cells on distinct, unbranched conidiophores, and acrogenous, solitary, non-septate conidia with schizolytic conidial secession. These characters separate the genus from *Domingoella* Petr. & Cif. (Petrak & Ciferri 1932), *Brachysporiella* Bat. (Batista 1952), and *Endophragmiella* B. Sutton (Sutton 1973). Ten species are currently included in *Acrogenospora*, all discovered on dead branches, rotten wood, or submerged bamboo (Ellis 1971, 1972, Hughes 1978, Goh et al. 1998, Zhu et al. 2005, Hu et al. 2010). The species are distinguished primarily on conidial shape, size, and ornamentation (Hughes 1978, Goh et al. 1998, Zhu et al. 2005).

Of the known species, *Acrogenospora hainanensis* is most similar to *A. sphaerocephala* (Ellis 1971) and *A. verrucispora* Hong Zhu et al. (Zhu et al. 2005) in the production of spherical or subspherical conidia. However, the new species differs from *A. verrucispora* (verrucose conidia $15\text{--}33 \times 14\text{--}33 \mu\text{m}$) in its smaller smooth-walled conidia and from *A. sphaerocephala* ($19\text{--}21.5 \mu\text{m}$ diam., conidial scars $5\text{--}7 \mu\text{m}$ wide) in its smaller conidia and narrower conidial scars.

Acrogenospora gigantospora S. Hughes, New Zealand J. Bot. 16: 314, 1978. FIG. 2

Colonies on the natural substratum effuse, black, glistening, hairy. Mycelium mostly immersed, composed of branched, septate, pale brown, smooth-walled hyphae. Conidiophores distinct, single or in groups of 2–4, unbranched, erect, straight or slightly flexuous, septate, smooth, brown to dark brown, up to $265 \mu\text{m}$ long, $5.5\text{--}8.5 \mu\text{m}$ thick. Conidiogenous cells monoblastic, integrated, terminal, cylindrical, smooth, pale brown, with multiple percurrent extensions. Conidial secession schizolytic. Conidia solitary, acrogenous, dry, broadly obovoid to

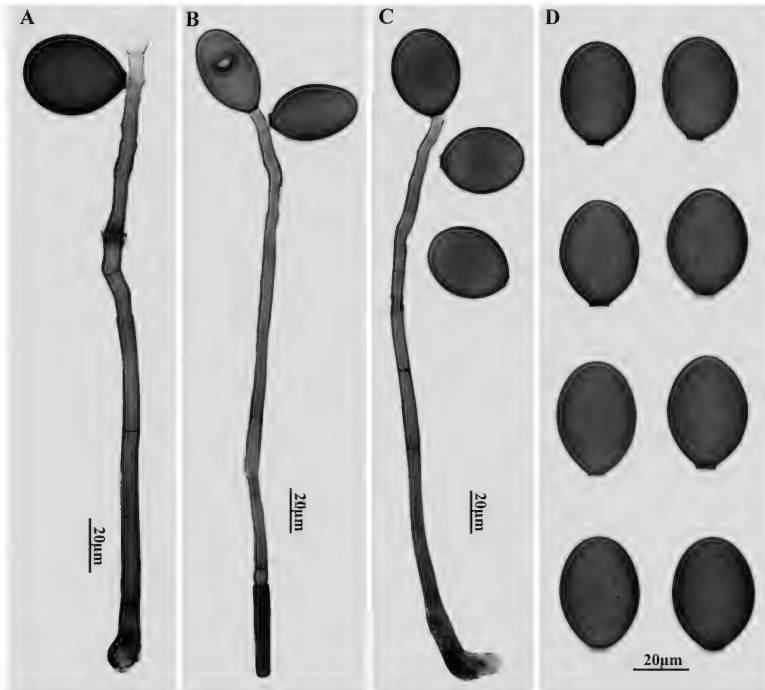


FIG. 2. *Acrogenospora gigantospora*. A–C. Conidiophores, conidiogenous cells with conidia. Note that in A and B the mature conidium is pushed sideways following successive percurrent extension of the conidiogenous cells. D. Conidia.

subspherical, smooth, dark brown to black, non-septate, $37\text{--}42 \times 24\text{--}30\ \mu\text{m}$, with a truncate base $5\text{--}7.5\ \mu\text{m}$ wide.

SPECIMEN EXAMINED: CHINA, GUANGDONG PROVINCE: Chebaling National Nature Reserve, on dead branches of unidentified broad-leaved tree, 19 Oct 2010, J. Ma (HSAUP H5402).

COMMENTS – *Acrogenospora gigantospora* is reported for the first time from China. Except for its shorter narrower conidiophores, our collection fits well with the original description of *A. gigantospora* (Hughes 1978; conidiophores $\leq 700 \times 9\text{--}14.5\ \mu\text{m}$). *Acrogenospora gigantospora* appears to be most closely related to *A. ovalis* Goh et al. (Goh et al. 1998) in conidial shape but differs in having larger conidia and a wider conidial scar. In addition, mature *A. gigantospora* conidia are brown to dark brown while those of *A. ovalis* are mid orange-brown.

Nakataea fusispora (Matsush.) Matsush., Icon. Microfung. Matsush. Lect.: 100, 1975. FIG. 3

= *Vakrabeeja fusispora* Matsush., Microfungi Solomon Is. Papua-New Guin.: 66. 1971.

= *Pyricularia fusispora* (Matsush.) Zucconi, Onofri & Persiani, Micol. Ital. 13(2): 9. 1984.

Colonies on the natural substratum effuse, dark brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, smooth-walled hyphae. Conidiophores conspicuous, mononematous, unbranched, straight or flexuous, smooth, septate, brown, paler towards the apex, $64\text{--}95\ \mu\text{m}$ long, $3.5\text{--}4.5\ \mu\text{m}$ thick. Conidiogenous cells polyblastic, integrated, terminal becoming intercalary, sympodially proliferating, denticulate, thin-walled; denticles subcylindrical, flat and open at the apex, septate at the base. Conidia solitary, dry, apical and lateral, verruculose, fusiform, acute at the apex, with a denticle at the base, 3-septate, the cell at each end hyaline or very pale brown, central cells pale to mid brown; conidia $22\text{--}34 \times 6\text{--}7.5\ \mu\text{m}$.

SPECIMEN EXAMINED: CHINA, HAINAN PROVINCE: tropical forest of Bawangling, on dead branches of unidentified broad-leaved tree, 11 Dec 2009, J. Ma (HSAUP H5125).

COMMENTS — *Nakataea*, established by Hara (1939) with *Helminthosporium sigmoideum* Cavara [= *N. sigmoidea* (Cavara) Hara] as the type species, is characterized by solitary, apical and lateral conidia seceding rhexolytically from polyblastic, integrated, terminal becoming intercalary, sympodially extending conidiogenous cells. The conidia are falcate, sigmoid, fusiform or obclavate, almost always 3-euseptate, versicolored, end cells paler than the central cells, with a distinct basal frill (Ellis 1971). *Vakrabeeja* Subram. (Subramanian 1956) is based on the same type species as *Nakataea* and therefore a superfluous homotypic synonym. Kirk (1983) suggested that *Nakataea* should be synonymised with *Pyricularia* Sacc., because their type species were morphologically similar and both had *Magnaporthe* teleomorphs. ITS rRNA

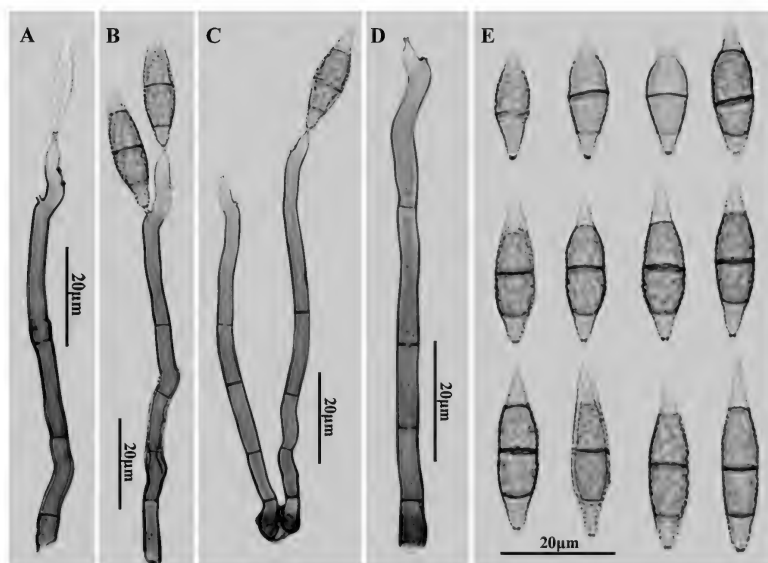


Fig. 3. *Nakataea fusispora*. A–C. Conidiophores, conidiogenous cells with denticles and conidia. D. Conidiophore and conidiogenous cell. E. Conidia.

sequence phylogenetic analyses (Bussaban et al. 2005) suggest that *N. fusispora* is distinct from *Pyricularia* species and should be maintained in *Nakataea*.

Nakataea fusispora is most similar to *N. sigmoidea* (Hara 1939) in conidial shape but can be separated from *N. sigmoidea* (conidia $40\text{--}83 \times 11\text{--}14\text{ }\mu\text{m}$, smooth) by its smaller conidia with verruculose walls. The conidia of our collection are somewhat wider than cited in the original description by Matsushima (1971; $4.8\text{--}6\text{ }\mu\text{m}$). The conidial length ranges of our and Matsushima's material overlap, and other morphological features of our fungus satisfy the original description of *N. fusispora*. This fungus is reported for the first time from China.

Pseudospiropes costaricensis (E.F. Morris) de Hoog & Arx, Kavaka 1: 59, 1974
[“1973”]

FIG. 4

= *Pleurophragmium costaricensis* E.F. Morris, Mycologia 64: 893. 1972.

Colonies on natural substratum effuse, brown, hairy. Mycelium partly superficial, partly immersed in the substratum, composed of branched, septate, smooth-walled hyphae. Conidiophores distinct, single or in groups, erect, unbranched, straight or flexuous, smooth, septate, brown to dark brown, paler toward the apex, $160\text{--}290\text{ }\mu\text{m}$ long, $6\text{--}9\text{ }\mu\text{m}$ wide. Conidiogenous cells holoblastic, polyblastic, indeterminate, terminal becoming intercalary, pale brown to brown, integrated, always with holoblastic sympodial extensions.

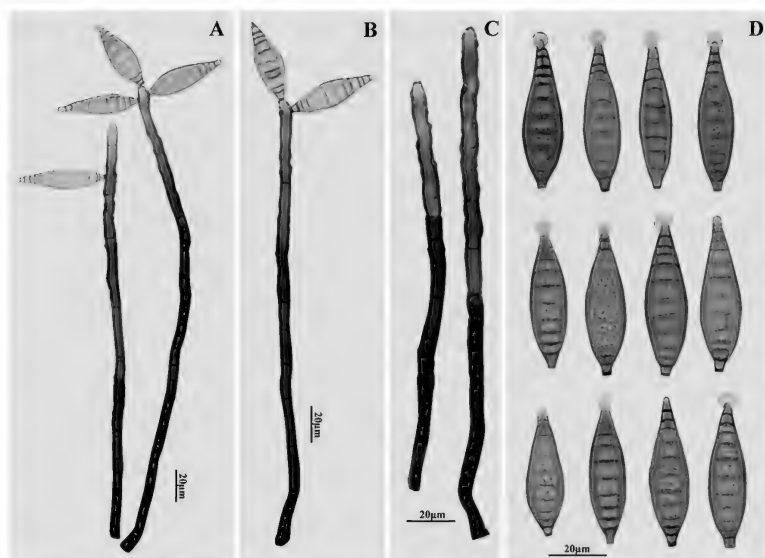


FIG. 4. *Pseudospiropes costaricensis*. A–B. Conidiophores and conidiogenous cells with conidia. C. Conidiophores and conidiogenous cells. D. Conidia.

Conidiogenous loci enlarged, thickened, protuberant and black, lenticular. Conidial secession schizolytic. Conidia apical and lateral, solitary, dry, simple, obclavate or fusiform, pale brown or brown, smooth, 8–11-distoseptate, $40\text{--}53 \times 11\text{--}14 \mu\text{m}$, apical cell with a hyaline vesicle, basal cell with a truncate base, $2\text{--}3.5 \mu\text{m}$.

SPECIMEN EXAMINED: CHINA, GUANGDONG PROVINCE: Chebaling National Nature Reserve, on dead branches of unidentified broad-leaved tree, 19 Oct 2010, J. Ma (HSAUP H5419).

COMMENTS – Since the establishment of *Pseudospiropes* M.B. Ellis, based on *Helminthosporium nodosum* Wallr. [$\equiv P. nodosus$ (Wallr.) M.B. Ellis], there have been numerous additions to the genus. However, Sinclair et al. (1997) pointed out that *Pseudospiropes* is heterogeneous. Castañeda et al. (2001) reviewed the genus and proposed three new segregate anamorphic genera — *Minimelanolocus* R.F. Castañeda & Heredia, *Nigrolentilocus* R.F. Castañeda & Heredia, and *Matsushimiella* R.F. Castañeda & Heredia— based on the morphology of conidiogenous loci, conidial septation, and conidial secession. As presently constituted, *Pseudospiropes* is characterized by polyblastic, integrated, terminal becoming intercalary, sympodial, cicatrized conidiogenous cells bearing broadly enlarged, thickened, often dark and protuberant scars, and solitary, apical and lateral, distoseptate conidia with schizolytic conidial secession (Ellis 1971, Castañeda et al. 2001). Currently, *Pseudospiropes* contains 13 valid species (Ma et al. 2011).

Pseudospiropes costaricensis was originally assigned to *Pleurophragmium* Costantin and subsequently transferred to *Pseudospiropes* by de Hoog & Arx (1974) due to its cicatrized conidiogenous cells with large, often dark and prominent scars. The conidia of *Pseudospiropes costaricensis* are morphologically similar to those of *P. hachijoensis* Matsush. (Matsushima 1975), *P. josserandii* (Bertault) Iturr. (Iturriaga & Korf 1990), *P. nodosus*, and *P. simplex* (Nees & T. Nees) M.B. Ellis (Ellis 1971). However, *P. costaricensis* can be easily separated from *P. hachijoensis*, *P. josserandii*, *P. nodosus* and *P. simplex* by its conidia, which have a hyaline vesicle at the apex. Except for having more conidial septa, our collection overlaps well with the original description of *P. costaricensis* (Morris 1972; 6–9 septa). This fungus is reported as new to the Chinese mycobiota.

Acknowledgments

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A new species of *Nectria* on *Populus* from ChinaZHAO-QING ZENG^{1,2} & WEN-YING ZHUANG^{1*}¹ State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China² Graduate University of Chinese Academy of Sciences, Beijing 100049, ChinaCORRESPONDENCE TO *: zhuangwy@im.ac.cn

ABSTRACT – A new species, *Nectria zangii*, is described on *Populus* branches from Donglingshan in western Beijing. The anatomy of perithecia, which become cupulate when dry, and positive reactions to KOH and lactic acid of the fungus indicates that this species belongs in the genus *Nectria*. It is characterized by small, non-septate, allantoid ascospores and small, subcylindrical to narrowly clavate asci. Sequence analysis of the combined nuclear ribosomal DNA ITS1-5.8S-ITS2 and partial β -tubulin gene confirm its taxonomic position in *Nectria* as a species new to science.

KEY WORDS – taxonomy, morphology

Introduction

The genus *Nectria* (Fr.) Fr. was established in 1849 and typified by *N. cinnabarina* (Clements & Shear 1931). About 34 species are currently accepted in the genus (Rossman et al. 1999, Kirk et al. 2001, Döbblers 2005a,b, Marincowitz et al. 2008, Pande 2008). Ten species are known from China (Teng 1963, Tai 1979, Wang et al. 1999, Zhuang & Zhang 2002, Zhang & Zhuang 2003a,b). The genus is characterized by well-developed stromata, subglobose, globose to ellipsoid, red to dark red, fleshy, soft-textured, uniloculate, warted perithecia that become cupulate when dry, and associated with coelomycetous anamorphs (Rossman et al. 1999, Hirooka et al. 2009). Ascospores of *Nectria* are variable and usually broadly ellipsoid to long-fusiform, hyaline to yellow-brown, smooth to striate, non- to multi-septate or muriform. Members of the genus are typically weak parasites of woody plants, and occur on hardwood trees and shrubs throughout temperate regions of the Northern Hemisphere (Samuels et al. 2009, Hirooka et al. 2011).

In this study, a new species of *Nectria* with small allantoid non-septate ascospores and subcylindrical narrow asci is described. On the basis of morphology and sequence analysis of nuclear ribosomal DNA ITS1-5.8S-ITS2

(ITS) and partial β -tubulin gene, its position in *Nectria* is confirmed, and its relationship with similar species of the genus is discussed.

Materials & methods

The taxonomic treatments and methods described in Luo & Zhuang (2010) were generally followed for morphological studies. Water was used as the mounting medium for microscopic examinations and measurements. Photographs were taken from water or lactophenol cotton blue mounts with a Canon G5 (Tokyo, Japan) digital camera connected to a Zeiss Axioskop 2 plus microscope (Göttingen, Germany). For anatomic studies, longitudinal sections through ascomata were made with a freezing microtome (YD-1508-III, Yidi Medical Appliance Factory, Jinhua, Zhejiang) at a thickness of ca. 8 μ m. Continuous measurements of individual structures are based on 30 measurements, except when otherwise noted. Single-spore isolates were obtained from recent collections. Colony characters were recorded from cultures on potato dextrose agar (PDA, Gams et al. 1998). Microscopic descriptions of the anamorphs were taken from cultures after 14 days. Specimens examined are deposited in the Mycological Herbarium, Institute of Microbiology, Chinese Academy of Sciences (HMAS), and cultures are kept in the State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences.

DNA was extracted from mycelium harvested from colonies on PDA after 2 weeks (Wang & Zhuang 2004). Sequences of ITS and the partial β -tubulin gene were amplified with primer pairs ITS5-ITS4 (White et al. 1990) and T1-T222 (O'Donnell & Cigelnik 1997) respectively. The PCR reaction mixture consisted of 2.5 μ l 10 $\times$ PCR buffer, 1.5 μ l MgCl₂ (25 mM), 1.25 μ l each primer (10 μ M), 0.5 μ l dNTP (10 mM each), 1.25 μ l DNA

TABLE 1. Materials used in this study.

SPECIES	COLLECTION NO.	ITS nrDNA GENBANK NO.	β -tubulin GENBANK NO.
<i>Cosmospora coccinea</i> Rabenh.	CBS 114050	HM484537	HM484589
<i>Nectria australiensis</i> Seifert	HMAS 83397	GU075855	HM054111
<i>N. berolinensis</i> (Sacc.) Cooke	CBS 126112	HM484543	HM484594
<i>N. cinnabarina</i> (Tode) Fr.	CBS 125150	HM484684	HM484820
	CBS 125158	HM484696	HM484830
<i>N. coryli</i> Fuckel	CBS 129156	HM484539	HM484596
<i>N. miltina</i> (Mont.) Mont.	CBS 121121	HM484547	HM484609
<i>N. nigrescens</i> Cooke	CBS 125148	HM484707	HM484806
	CBS 129360	HM484711	HM484808
<i>N. pseudocinnabarina</i> Rossman	CBS 128673	HM484553	HM484608
<i>N. pseudotrichia</i> (Schwein.) Berk. & M.A. Curtis	HMAS 183559	HM054154	HM054115
	HMAS 183175	HM054138	HM054114
<i>N. zangii</i>	HMAS 251258	JN997424 ^a	JN997421
	holotype, HMAS 251247	JN997425	JN997422
	HMAS 188502	JN997426	JN997423
<i>Neonectria ramulariae</i> Wollenw.	CBS 151.29	HM054150	HM054124

^a Numbers in bold = newly submitted sequences.

template, 0.25 µl Taq polymerase (5 U/µl) and 16.5 µl ddH₂O. Reactions were performed on an ABI 2720 Thermal Cycler (Gene Co. Ltd.) with cycling conditions of denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C (ITS region) and at 55°C (β-tubulin gene) for 30 s and elongation at 72°C for 45 s, with a final extension step at 72°C for 10 min to complete the reactions. PCR products were purified with the PCR Product Purification Kit (Bicolor BioScience & Technology Co.) and sequenced with the ABI BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI 3730 XL DNA Sequencer (SinoGenoMax Co. Ltd.). ITS5 and ITS4 (for ITS), and T1 and Bt2b (for the partial β-tubulin gene) were employed as sequencing primers (White et al. 1990, Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). Sequences of related species were retrieved from GenBank and are listed in TABLE 1.

All sequences were aligned using ClustalX 1.8 (Thompson et al. 1997), and the alignments were visually adjusted by BioEdit 7.0.5 (Hall 1999) when necessary. A partition homogeneity test was performed with 1000 replicates in PAUP 4.0b10 (Swofford 2002) to evaluate statistical congruence between sequence data from ITS and β-tubulin gene regions. The partition homogeneity test ($P = 0.01$) suggested that the individual partitions were congruent (Farris et al. 1995, Cunningham 1997). A neighbor-joining tree was generated using MEGA 4.10 (Tamura et al. 2007) based on combined sequences of ITS and partial β-tubulin gene with *Cosmospora coccinea* and *Neonectria ramulariae* as the outgroup taxa. The Kimura 2-parameter was selected as the nucleotide substitution model, and gaps or missing data were pairwise deleted. Bootstrap analyses were performed with 1000 replicates to test phylogeny branch support.

Taxonomy

Nectria zangii Z.Q. Zeng & W.Y. Zhuang, sp. nov.

FIGURE 1

MYCOBANK 563722

Differs from *Nectria miltina* by thinner perithecial walls, narrower asci, smaller ascospores, and occurrence on twigs of *Populus* sp.

TYPE: China. Beijing, Donglingshan, alt. 1150 m, on branches of *Populus* sp., 20 July 2011, Z.Q. Zeng & H.D. Zheng 7684 (Holotype, HMAS 251247).

ETYMOLOGY: The specific epithet honors the late Chinese mycologist, Prof. M. Zang.

Ascomata perithecial, gregarious, up to 40 in a group, with a well-developed stroma that is erumpent through bark, superficial, subglobose to globose, 168–200 µm high, 151–203 µm diam., becoming cupulate upon drying, red when fresh and reddish brown when dry, turning dark red in 3% KOH aqueous solution and orange-red to orange in lactic acid, surface somewhat roughened when dry; with warts or a irregular covering layer of various thickness, 0–32 µm high, cells angular, 3.5–15 × 2.5–5 µm, wall 1–1.5 µm thick. Ascomatal wall 16–26 µm thick, of two layers; outer layer 10–16.5 µm thick, cells angular, 2–7 × 3–6 µm, wall 1–1.5 µm thick; inner layer 5–10.5 µm thick, cells flattened, 9.5–15.5 × 1.5–3.5 µm, wall 1–1.5 µm thick. Asci subcylindrical to narrowly clavate, 8-spored, with an apical ring, 28–35 × 2.5–3 µm ($n = 50$), biseriate

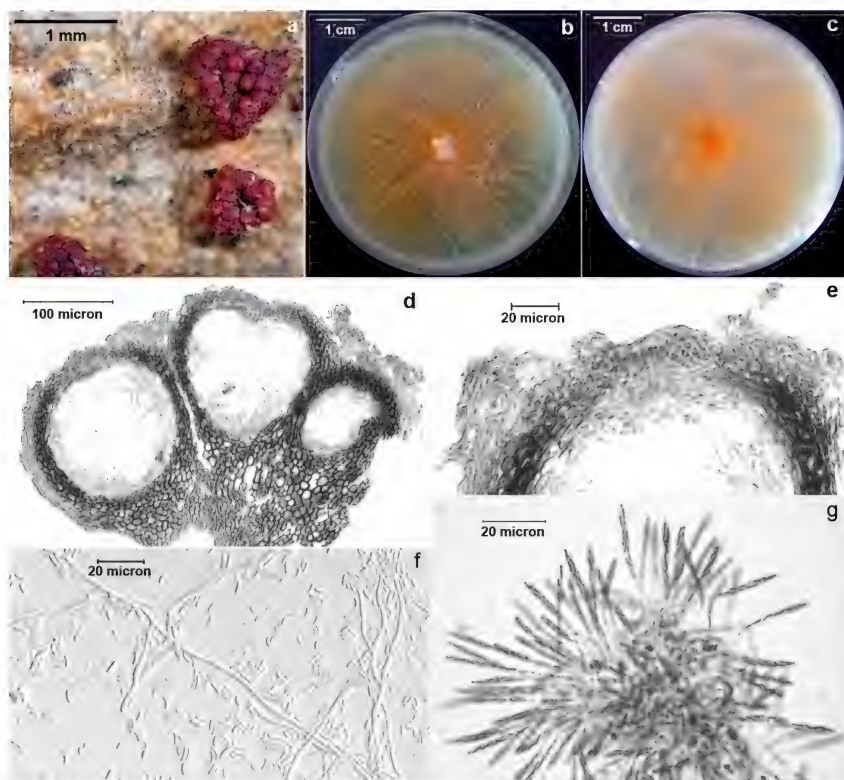


FIG. 1. *Nectria zangii* (holotype, HMAS 251247). a. Ascomata on the natural substrate. b. Colony on PDA, obverse. c. Colony on PDA, reverse. d. Median section of ascomata. e. Structure of ascoma at apical portion. f. Asci with ascospores. g. Conidiophores and conidia.

to irregularly biseriate. Ascospores allantoid to rod-shaped, straight to slightly curved, non-septate, hyaline, smooth, $3.5\text{--}5.5(-6) \times 0.9\text{--}1.2(-1.4) \mu\text{m}$ ($n = 50$).

In culture, colonies reaching 57 mm in diameter after 7 days on PDA at 25°C under daylight, aerial mycelium sparse to absent, yellowish, submerged mycelia forming a root-like or vein-like structure, reverse light orange. Conidiophores unbranched to sparsely branched, septate, $29\text{--}79 \times 2\text{--}3.2 \mu\text{m}$, arising from agar surface throughout the colony, dominating near the margin. Microconidia allantoid to rod-shaped, not or slightly curved, hyaline, aseptate, $3\text{--}6 \times 0.8\text{--}1 \mu\text{m}$ ($n = 50$). Macroconidia and chlamyospores not observed.

ADDITIONAL SPECIMENS EXAMINED: CHINA. BEIJING, Donglingshan, alt. 1150m, on rotten branch of *Populus* sp., 20 July 2011, Z.Q. Zeng & H.D. Zheng 7648–7651 (HMAS 188501–188504); 7652 (HMAS 251248); 7653–7654 (HMAS 188505–188506); 7655 (HMAS 251249); 7656 (HMAS 188507); 7657–7660 (HMAS 251250–251253);

7663 (HMAS 188508); 7665–7667 (HMAS 188509–188511); 7668 (HMAS 251254); 7669–7671 (HMAS 188512–188514); 7672 (HMAS 251255); 7673–7680 (HMAS 188515–188522); 7681 (HMAS 251256); 7682 (HMAS 188523); 7683 (HMAS 251257); 7685–7694 (HMAS 188524–188533); 7695 (HMAS 251258); 7696–7698 (HMAS 188534–188536).

COMMENTS – Morphologically, the perithecial anatomy, perithecia becoming cupulate when dry, and positive reaction to KOH and to lactic acid indicates the new species' position in *Nectria*. Among the existing species of the genus, *N. zangii* is most similar to *N. miltina* in shape of asci and ascospores. *Nectria miltina*, however, differs in having a thicker perithecial wall (25–35 µm thick), wider asci (28–36 × 3.5–5 µm), larger ascospores (5.5–7 × 1.5–2 µm), and an entirely different host. *Nectria miltina* occurs on leaves of the monocotyledonous plant family *Agavaceae* (Rossman et al. 1999) instead of on dicotyledonous *Populus* sp.

Sequence analysis of the combined ITS and partial β-tubulin gene from *N. zangii* and eight other *Nectria* species with various ascospore shapes and septation confirms taxonomic position of the new species (FIG. 2). Our results support two major distantly related clades within the sampled *Nectria* species. *Nectria zangii*, *N. berolinensis*, *N. miltina*, *N. coryli*, and *N. pseudocinnabarina* group together with high bootstrap support (99%). The latter four species were reported having sporodochial anamorphs (Rossman et al. 1999). The other

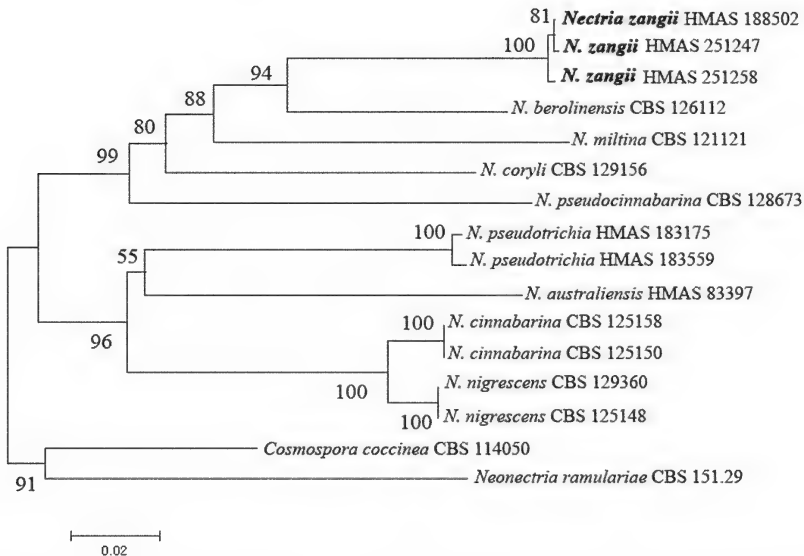


FIG. 2. Neighbor-joining tree based on combined sequences of ITS and partial β-tubulin gene showing the relationships among some *Nectria* species.

sampled species, including the type species *N. cinnabarina*, group together with 96% bootstrap support (FIG. 2). These results are identical to those by Hirooka et al. (2011). *Nectria berolinensis*, shown as sister to *N. zangii* with 94% bootstrap support, differs in larger perithecia (250–300 µm diam.) and 5–9-septate larger (16–20 × 7–8 µm) muriform elliptical ascospores (Seaver 1909). *Nectria miltina* also groups with *N. berolinensis* and *N. zangii* with relatively high support (88%). Thus, both morphology and DNA sequence analysis support the recognition of *N. zangii* as a new species.

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MYCOTAXON

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A new species of the lichen genus *Phlyctis* from Maharashtra, IndiaGAYATRI CHITALE¹ & URMILA MAKHIJA²*Agharkar Research Institute, G.G. Agarkar Road, Pune -411 004, India*CORRESPONDENCE TO: ¹*gayatri4u@gmail.com* & ²*uv_makhija@hotmail.com*

ABSTRACT—A new species, *Phlyctis communis*, characterized by 8-spored asci, 7–14(–16) transversely septate ascospores, and salazinic and norstictic acids, is described from India.

KEY WORDS—*Ascomycetes*, *Ostropales*, taxonomy

Introduction

Although taxonomic accounts of several lichen genera from Maharashtra have been published and many species have been described (Chitale et al. 2008, 2009, 2011, Chitale & Makhija 2008, Dube et al. 2005, Dube & Makhija 2008, 2010), one locally common and abundant interesting species has remained unpublished. For several years a conspicuously grayish-white unidentified crustose lichen was known to occur all over Maharashtra, mostly during the monsoons in the humid high altitude regions. This has now been identified and is described as a new species of the genus *Phlyctis*.

The subtropical to temperate lichen genus *Phlyctis* (Wallr.) Flot., confirmed by phylogenetic sequence analysis as belonging in the *Ostropales* (Miadlikowska et al. 2006), is characterized by a smooth to verrucose crustose thallus, protococcoid green algal photobiont, sunken or erumpent, round apothecioid ascomata with indistinct thalline margins, proper exciple present or absent, unbranched or apically furcate paraphyses, 1–8-spored asci, and ascospores that are colourless, transversely septate to multicelled-muriform, thin walled, elongate-ellipsoid, and non-halonate.

Phlyctis species generally contain one or several of the following depsidone acids: stictic, constictic, norstictic, connorstictic, hypostictic, salazinic psoromic, neopsoromic and/or protocetraric acids (Galloway & Guzmán 1988).

The genus is widely distributed, with 12 species listed by Kirk et al. (2008) and five others from Great Britain and Ireland (Benfield et al. 2009), China (Ma

et al. 2010), India (Joshi et al. 2010), and Australia (Lumbsch et al. 2011) added since 2008. Of these only five — *Phlyctis himalayensis* (Nyl.) D.D. Awasthi, *P. nepalensis* Räsänen, *P. polyphora* Stirt., *P. karnatakana* S. Joshi & Upreti, and *P. subagelaea* S. Joshi & Upreti — have been reported from the Indian subcontinent (Awasthi 2000, Joshi et al. 2010).

In the present study, one new species has been discovered from Maharashtra that represents the first record of *Phlyctis* from this area.

Materials & methods

The specimens were examined using a stereomicroscope and a light microscope. Sections of the thalli and apothecia were stained with Lugol's iodine solution. All sections examined were mounted in LPCB (lactophenol with cotton blue). TLC protocols followed Culberson & Kristinsson (1970) and White & James (1985). All specimens were observed under UV light (365 nm). The present study is based on the material preserved in the lichen herbarium of Ajarekar Mycological Herbarium (AMH) and recent collections by the authors from Maharashtra state.

Taxonomy

Phlyctis communis Chitale & Makhija, sp. nov.

FIGS 1–4

MYCOBANK MB 563474

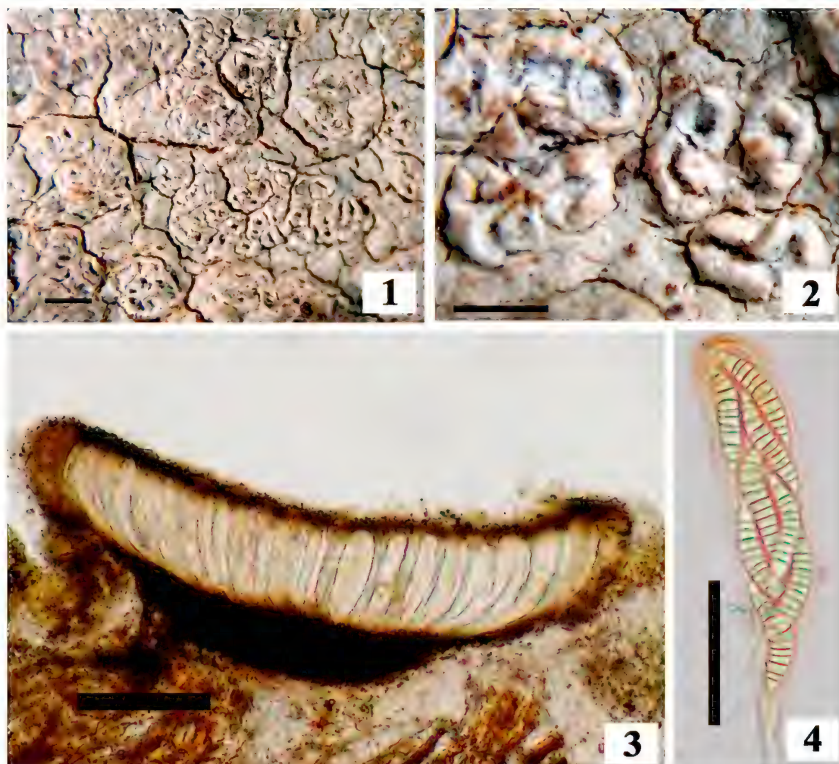
Similis Phlyctidae karnatakanae sed *ascosporis* 7–14(–16) *trans-septatis et acidum norsticticum et salazinicum continenti differt.*

TYPE: India, Maharashtra, Satara District, Mahabaleshwar, 24.9.1997, U.V. Makhija, (Holotype, AMH 97.52).

ETYMOLOGY: Latin *communis*, referring to the plentiful or common occurrence.

THALLUS crustose, corticolous, grayish or greenish-white, spreading over the bark substratum in 5–12 cm patches, cracked, areolate, matt, sometimes glossy, white in cracks and 90–120 µm thick at the margin; epiphloeodal algal layer 30–35 µm thick, green algae single celled, more or less globose, 5–7 µm diam.; hypothallus black.

ASCOMATA black, round, oblong, numerous, scattered all over the thallus and radially arranged in small 1–2(–3.5) mm diam. circles, later grouped and merging into each other forming patches on the bark of tree trunks, individual ascomata ≤1 mm across, immersed in the thallus; disc black, plane to concave, covered by a white pruina; exciple brownish, composed of loosely interwoven hyphae in the peripheral region, and blackish-brown at the base; epihymenium dark brown, 30–33 µm thick, KI–; hymenium colourless to light yellow, 72–81 µm tall, KI+ blue; paraphyses simple to branched at tips; hypothecium brownish, 12–15 µm thick; asci 8-spored, 90–105 × 18–24 µm, entire ascus KI+ blue. Ascospores hyaline, 7–14(–16) transversely septate, very rarely with 1–2 vertical septa, 18–33(–45) × 6–9 µm.



FIGURES 1–4. *Phlyctis communis* (holotype): 1–2, habit; 3, vertical section of the ascocarp; 4, transseptate ascospores. Scale bars: 1–2 = 1 mm; 3–4 = 50 µm.

CHEMISTRY—Thallus K⁺ yellow turning red, C[–], KC[–], P⁺ yellow; UV[–]; norstictic and salazinic acids present.

SELECTED ADDITIONAL SPECIMENS EXAMINED (total specimens = 110, all in AMH)—**INDIA. MAHARASHTRA:** KOLHAPUR DISTRICT, Ajra, 6.10.2004, B.C. Behera, N. Verma, AMH 04.320, U.V. Makhija & G.S. Chitale, AMH 04.389, Amba, 16.10.1974, C.R. Kulkarni & A.V. Prabhu, AMH 74.1282, on the way to Kumbhi from Gaganbawada, 12.10.2000, B.A. Adawadkar & K.R. Randive, AMH 00.337. **PUNE DISTRICT,** Ambay valley, 10.2.2006, G.S. Chitale, AMH 06.181; Boma Hills, Khandala, 19.9.1974, M.B. Nagarkar & C.R. Kulkarni, AMH 74.691, Walwan Dam, 16.9.2002, A.V. Bhosale & G.S. Chitale, AMH 02.113. **SATARA DISTRICT,** Mahabaleshwar, 1.11.1973, C.R. Kulkarni, AMH 73.2938; Panchgani, Tata Holiday Home, 29.9.2003, U.V. Makhija & B.C. Behera, AMH 03.369. **SINDHUDURG DISTRICT,** Ajra to Amboli, 7.12.1974, P.G. Patwardhan & A.V. Prabhu, AMH 74.2248, 10.10.2000, U.V. Makhija & V.A. Mantri, AMH 00.176.

REMARKS— With respect to external morphology and ascospore size, *Phlyctis communis* resembles *P. karnatakana*, also from India, which differs in fewer ascospore septa (7 transsepta) and containing only norstictic acid.

Phlyctis subuncinata Stirt., also with norstictic acid and transversely septate ascospores, differs in having a sorediate thallus.

Two other *Phlyctis* species with transversely septate ascospores — *P. himalayensis* from India and *P. longifera* (Nyl.) D.J. Galloway & Guzmán from New Zealand — differ especially in having larger, 7-septate ascospores. *Phlyctis himalayensis* ascospores measure $60\text{--}75 \times 6\text{--}8 \mu\text{m}$, whereas *P. longifera* ascospores are $55\text{--}86 \times 5\text{--}7 \mu\text{m}$.

Phlyctis psoromica Elix & Kantvilas, also with transversely septate ascospores, has only (3–)7-septate ascospores ($30\text{--}39.3\text{--}50(\text{--}52) \times 4\text{--}5.2\text{--}6 \mu\text{m}$) and contains psoromic acid.

The somewhat similar *P. polyphora* from India shares a whitish thallus and a pruinose ascomatal disc ≤ 1 mm diam. but is distinguished by very large ($60\text{--}110 \times 7.5\text{--}9.5 \mu\text{m}$) muriform ascospores.

Phlyctis communis is very common and has been collected from semi-evergreen to dry deciduous forests with relative humidity between 15–90% where thalli grow in huge patches on the bark of *Ficus benghalensis* and *Casuarina equisetifolia* where it frequently associates with *Arthothelium awasthii* Patw. & Makhija.

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MYCOTAXON

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Two new freshwater species of *Annulatascaceae* from ChinaDIAN-MING HU^{1,2}, LEI CAI^{1*}, ALI HASSAN BAHKALI³ & KEVIN D. HYDE^{2,3,4,5*}¹State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China²International Fungal Research & Development Centre, The Research Institute of Resource Insects, Chinese Academy of Forestry, Bailongsi, Kunming 650224, China³Botany and Microbiology Department, College of Science, King Saud University, Riyadh, Saudi Arabia⁴School of Science, Mae Fah Luang University, Chiang Rai, Thailand⁵Institute of Excellence for Fungal Research, Mae Fah Luang University, Chiang Rai, Thailand* CORRESPONDENCE TO: mrcailei@gmail.com, kdhyde3@gmail.com

ABSTRACT — Two new species belonging to *Annulatascaceae* are described and illustrated on submerged wood from a freshwater stream. *Annulatascus menglensis*, differentiated from other *Annulatascus* species by its hyaline ascomatal neck, is also characterized by cylindrical asci with a large bipartite refractive J– apical ring and hyaline fusiform ascospores without sheath or appendages. *Aqualignicola vaginata*, which is distinguished from *A. hyalina* in producing a gelatinous sheath surrounding the unicellular hyaline ascospores, is further characterized by its membranous ascomata with lanceolate setae that surround the neck, long cylindrical asci with a relatively large apical ring.

KEY WORDS — aquatic, *Ascomycota*, fungi, taxonomy

Introduction

Freshwater fungi are an important ecological group because of their indispensable function as decomposers in freshwater ecosystems (Wong et al. 1998a); they also have potential application in producing various and useful bioactive secondary metabolites (Bucher et al. 2004, Zhang et al. 2008, Hosoe et al. 2010, Dong et al. 2011). Freshwater fungi are however, relatively poorly investigated comparing to their terrestrial counterpart. Until recently, freshwater fungi had been reported mainly from Europe, North America, and Southeast Asia (Cai et al. 2006b, Raja et al. 2009). Although China has a high biodiversity of fungi and abundant freshwater resources, investigation of freshwater fungi has been mostly restricted to Hong Kong and Yunnan Province.

Annulatascaceae S.W. Wong et al. comprises many freshwater genera (Vijaykrishna et al. 2006). The family is characterized morphologically by dark

brown or black ascomata with ostiolate necks, long cylindrical unitunicate asci with a relatively massive, refractive apical ring, and uniseriate, hyaline to brown, fusiform or ellipsoidal ascospores (Wong et al. 1998b, Ho & Hyde 2000). Seventy-five species representing 21 genera are currently placed in *Annulatascaceae* (Kirk et al. 2008).

Our research group has focused on investigating the diversity of freshwater fungi in China for over ten years (Hyde et al. 2000, Luo et al. 2004, Cai et al. 2005, 2008, Hu et al. 2007, 2010a, b). During our continuing investigation, two undescribed ascomycetes belonging to the *Annulatascaceae* were collected on submerged wood and are described and illustrated in this paper.

Materials & methods

D.M. Hu, who has investigated freshwater ascomycetes on submerged woody material in the southern provinces of China (including Guizhou, Jiangxi and Yunnan provinces) since 2008, has collected 875 samples from streams, lakes, ditches, and ponds. After return to the laboratory, the samples were incubated following Cai et al. (2006a). Samples were examined for fungal fruiting bodies under a dissecting microscope (Leica MZ16A) during incubation. Observations and photographs were prepared from materials mounted in water under a compound microscope (Nikon E800). Single spore isolation as outlined by Choi et al. (1999) was attempted but unsuccessful. Herbarium specimens of the fungal taxa described in this study are deposited in the International Fungi Research and Development Centre (IFRDC).

Taxonomy

Annulatascus menglensis D.M. Hu, L. Cai & K.D. Hyde, sp. nov.

PLATE 1

MYCOBANK MB 563810

Differs from other *Annulatascus* species by its hyaline neck.

TYPE: China, Yunnan Province, Mengla, Wudaoban Stream, 21°24'N 101°36'E, alt: 660 m, 3 April 2009, D.M. Hu (*Holotype*, IFRDC 023-002).

ETYMOLOGY: *menglensis* refers to the collecting site, Mengla, a county in southern China.

ASCOMATA 180–200 high, 120–150 µm in diam., subglobose to ellipsoidal, superficial, solitary, ostiolate, black, coriaceous. NECK 150–340 × 40–60 µm, cylindrical, hyaline, membranous, periphysate. PERIDIUM composed of compressed cells, outer layers dark brown, inner layers hyaline. PARAPHYSES ca. 5–6 µm wide, broad at the base and tapering towards the apex, hyaline, septate, smooth-walled, simple, longer than asci. ASCI 115–163 × 10–13 µm, 8-spored, cylindrical, unitunicate, persistent, pedicellate, with a large bipartite, refractive, J- apical ring, ca. 3 µm high, 4 µm in diam. ASCOSPORES 21–25 × 8–10 µm (mean = 23.8 × 8.5 µm, n = 30), uniseriate, overlapping, fusiform, with acute ends, hyaline, aseptate, guttulate, smooth, thin-walled, without sheaths or appendages.



PLATE 1. *Annulatascus menglensis* (from holotype). a. Ascoma on submerged wood. b. Squash mount of an ascoma. c. Peridium comprising angular cells in surface view. d. Paraphyses. e–f. Asci. g. Upper part of an ascus showing the apical ring. h–k. Ascospores. Scale bars: a = 100 μ m, b = 50 μ m, c–d = 5 μ m, e–f = 20 μ m, g–k = 5 μ m.

HABITAT & DISTRIBUTION— Saprobic on submerged wood in fresh water in China.

ADDITIONAL SPECIMENS EXAMINED: CHINA. YUNNAN PROVINCE: Mengla, Wudaoban Stream, 21°24'N 101°36'E, alt. 660 m, 3 April 2009, D.M. Hu (IFRDSC 002-017, IFRDSC 002-018).

COMMENTS — *Annulatascus menglensis* has black, coriaceous ascomata and cylindrical asci with a large bipartite, refractive, J– apical ring. It fits well within the generic concept of *Annulatascus* (Hyde 1992). Thus far, 14 species have been included in the genus (Tsui et al. 2002, Barbosa et al. 2008, Abdel-Wahab et al. 2011). The new species resembles *A. velatisporus* K.D. Hyde (Hyde 1992) in having fusiform and aseptate ascospores. However, *A. velatisporus* differs from *A. menglensis* in having larger ascospores ($26\text{--}42 \times 9\text{--}12 \mu\text{m}$) with a gelatinous sheath. *Annulatascus menglensis* shares similar ascospore dimensions with *A. fusiformis* K.D. Hyde & S.W. Wong (Hyde & Wong 2000), *A. joannae* K.M. Tsui et al. (Tsui et al. 2002), *A. citriosporus* J. Fröhl. & K.D. Hyde (Fröhlich & Hyde 2000), and *A. apiculatus* F.R. Barbosa & Gusmão (Barbosa et al. 2008) but lacks their black necks and mucilaginous sheaths and/or appendages. *Annulatascus lacteus* K.M. Tsui et al. (Tsui et al. 2002), which also has aseptate ascospores without sheath or appendage, differs from *A. menglensis* in having a milky ascomata.

Aqualignicola vaginata D.M. Hu, L. Cai & K.D. Hyde, **sp. nov.**

PLATE 2

MYCOBANK MB 563811

Differs from *Aqualignicola hyalina* in having a hyaline ascomatal neck and a gelatinous sheath surrounding the ascospores.

TYPE: China, Yunnan Province, Mengla, Wudaoban Stream, 21°32'N E101°29'E, alt. 620 m, 2 April 2009, D.M. Hu (**Holotype**, IFRDC 021-043).

ETYMOLOGY: *vaginata* (= sheath) refers to the mucilaginous sheath surrounding the ascospore.

ASCOMATA 100–140 μm diam, globose, membranous, brown to black-brown, submerged, solitary. **NECK** 40–60 μm diam, 200–350 μm long, cylindrical, hyaline, periphysate, covered with setae; setae $15\text{--}35 \times 4\text{--}5 \mu\text{m}$, lanceolate, solid, surrounding the neck. **PERIDIUM** 7–14 μm thick, in surface view of *textura angularis*, composed of 5 layers of light-brown, pseudoparenchymatous cells. **PARAPHYSES** 4–6 μm in diam, 85–100 long, septate, flexuose, hypha-like, numerous, tapering towards the apex, embedded in a gelatinous matrix. **ASCI** $145\text{--}156 \times 5.5\text{--}6.5 \mu\text{m}$, 8-spored, cylindrical, pedicellate, unitunicate, apically truncate, with a large, J–, refractive, apical ring. **ASCOSPORES** $11\text{--}15 \times 5\text{--}6 \mu\text{m}$ (mean = $13.4 \times 5.3 \mu\text{m}$, $n = 50$), uniseriate, ellipsoidal-fusiform, hyaline, aseptate, thin-walled, multiguttulate, with a fan-shaped, hyaline mucilaginous sheath surrounding one end of the ascospores.

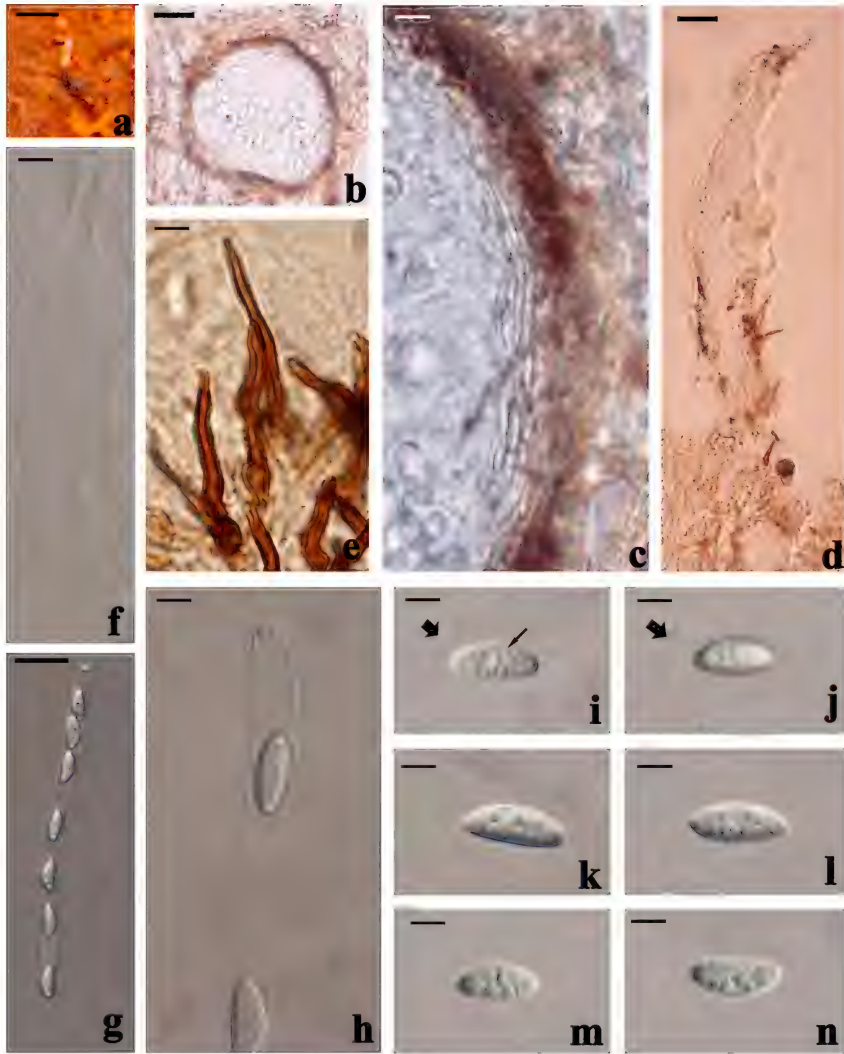


PLATE 2. *Aqualignicola vaginata* (from holotype). a. Ascoma on submerged wood. b. Section of an ascoma. c. Peridium. d. Section through a neck. e. Hair on neck. f. Paraphyses. g. Ascus. h. Upper part of an ascus, show the apical ring. i–n. Ascospores (arrows show the mucilaginous sheath and the multiple guttules). Scale bars: a = 100 μ m, b = 30 μ m, c = 5 μ m, d = 30 μ m, e, f = 5 μ m, g = 20 μ m, h–n = 5 μ m.

HABITAT & DISTRIBUTION — Saprobic on submerged wood in fresh water in China.

ADDITIONAL SPECIMENS EXAMINED: CHINA. YUNNAN PROVINCE: Mengla, Wudaoban Stream, 21°32'N 101°29'E, alt. 620 m, 2 April 2009, D.M. Hu (IFRDSC 004-003, IFRDSC 004-004, IFRDSC 004-005).

COMMENTS — Previously monotypic, *Aqualignicola* V.M. Ranghoo et al. is characterized by membranous ascomata with a neck surrounded by lanceolate setae, long cylindrical asci with a relatively large apical ring, and hyaline unicellular ascospores (Ranghoo et al. 2001). The new species fits well with the generic concept of *Aqualignicola* but differs from the type species, *A. hyalina*, in having a hyaline ascomatal neck and a mucilaginous sheath surrounding the ascospore. Furthermore, the ascospores and asci of *A. vaginata* are narrower than those of *A. hyalina* (ascospores: 14–15 × 6.25–7.5 µm; asci 9–12 µm diam.). *Aqualignicola vaginata* is also similar to species of *Aquaticola* W.H. Ho et al. (Ho et al. 1999) and *Longicollum* Zelski et al. (Zelski et al. 2011a). However, the species of the latter two genera have glabrous ascomata and lack the lanceolate setae surrounding the neck found in *A. vaginata*. *Aqualignicola vaginata* can be compared to the newly described *Chaetorostrum quincemilense* Zelski et al. (Zelski et al. 2011b) in that both taxa have a hyaline neck covered with setae; however the ascospores of *A. vaginata* are aseptate and much smaller than those of *C. quincemilense* (1–3-septate; 30–38 × 10–12 µm).

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**New records of little-known species of *Carbomyces*
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ABSTRACT— The genus *Carbomyces* is reported for the first time for the Mexican mycobiota. *Carbomyces emergens*, *C. gilbertsonii*, and *C. longii* were collected in the Chihuahuan desert, Mexico. *Carbomyces emergens* was more frequently collected and more widely distributed, while *C. gilbertsonii* and *C. longii* each have a restricted distribution and represent second records worldwide. The phenology, chorology, and macro-/microscopic characters (including spore ornamentation by SEM) are outlined for all species.

KEY WORDS— *Pezizales*, taxonomy, truffles

Introduction

Several collections of hypogeous and semi-hypogeous species have been recorded in recent studies of gasteroid and secotiid fungi in Chihuahua State, including the central Chihuahuan desert, Mexico (Lizárraga et al. 2010; Moreno et al. 2010). Fungi reported here were found on the soil surface and wrongly considered *in situ* to represent basidiomycetes. However, careful examination revealed that these fungi represent two different ascomycete species in the genus *Carbomyces* Gilkey.

Hypogeous ascomycetes, which are widely distributed, occur in different forest types (e.g., coniferous dominated by *Pinaceae*, angiospermous with *Betulaceae*, *Salicaceae*, *Fagaceae*, and *Myrtaceae*). Some even associate with herbaceous plants and shrubs, such as those in the *Cistaceae*. Cistaceous plants found in arid areas of the Mediterranean basin, southern Europe, northern

Africa, and the Middle East play an important role in plant conservation by protecting sensitive areas from erosion from heavy rain and human activity. In these areas, cistaceous plants can form mycorrhizal associations with several different fungal genera such as *Choiromyces*, *Picoa*, *Terfezia*, *Tirmania*, and *Tuber*.

However, in America, *Cistaceae* does not occur and arid lands are commonly dominated by *Cactaceae*, *Fouquieriaceae*, *Mimosaceae*, and *Zygophyllaceae*. *Carbomyces* was described from deserts of New Mexico and California (USA) for *C. emergens* and *C. longii* by Gilkey (1954), who published macro- and microscopical drawings of both species (Gilkey 1955).

Trappe & Weber (2001) later described in detail those two species and transferred a third species, *C. gilbertsonii*, into *Carbomyces* from the puffball basidiomycete genus *Abstoma* G. Cunn. where it had previously been misplaced (Zeller 1944). These truffles appear endemic to North American deserts, occurring in the Chihuahuan, Sonoran, and Mohave deserts from New Mexico to southern California, where they appear from September through April. *Carbomyces*, which is adapted to extreme xeric conditions, differs from other hypogeous ascomycetes in globose emergent ascomata, a two-layered peridium, and a powdery gleba at maturity resulting from the disintegrated asci (a feature also shared with *Elaphomyces* spp. and *Ruhlandiella berolinensis* Henn.).

Most specimens have been found *ex situ* since they are easily wind-blown when mature (lying dry and loose) or eaten and transported (when immature?) by rodent mycophagists (Zak & Whitford 1986), so that fungal populations can be easily intermixed and subsequently confused. Nevertheless, in the Chihuahuan desert (Mexico), we found pure (i.e. non-intermixed) populations *in situ* that appeared macroscopically very similar, to other known species based on spore ornamentation (smooth vs. rough) and shape (ellipsoid vs. globose).

Gilkey (1954) placed *Carbomyces* in the *Terfeziaceae*, but Trappe (1971) erected the monotypic *Carbomycetaceae* for the genus, a taxonomic position later accepted by several authors (Trappe 1979, Trappe & Weber 2001, Lumbsch & Huhndorf 2007). Nevertheless, the true relationship between *Carbomyces* and other ascomycetes was unknown until recently (Hansen & Pfister 2006). The putatively non-mycorrhizal genus *Carbomyces* is thought to be ectomycorrhizal (ECM), although no ECM hosts have been identified in its natural habitat (Trappe 1971; Zak & Whitford 1986; Trappe & Weber 2001).

In the present work all three *Carbomyces* species —*C. emergens*, *C. gilbertsonii*, *C. longii*— are reported from Mexico for the first time and redescribed from recently collected specimens.

Materials & methods

The ascocarps were studied *in situ* following general methods (Cifuentes et al. 1986, Moreno & Manjón 2010). Dried material was studied at the lab using KOH 5%,

Melzer's reagent (MLZ), Cotton blue (CB), Congo red 1%, and Hoyer's medium for microscopical observations. A Nikon Eclipse 80i phase-contrast microscope was used for spore measurements (on dead dried material) under oil immersion including surface structures such as spines or warts (on 25 spores).

Spore ornamentation was also studied under a scanning electron microscope Zeiss DSM 950. Critical point drying technique was performed on the samples prior to mounting, following Moreno et al. (1995).

The collections are kept in the Herbarium UACJ of the "Departamento de Ciencias Básicas, Universidad Autónoma de Ciudad Juárez," Mexico, with duplicate sets in Herbarium AH of the Universidad de Alcalá de Henares and the macromycetes collection of the Centro de Estudios Superiores del Estado de Sonora (CESUES).

High-quality DNA extract could be obtained from only one fruiting body of *Carbomyces emergens* (AH 39344), probably due to the extreme climatic conditions and high temperatures over long periods to which the other samples were naturally exposed. DNA extraction and PCR protocols followed Checa et al. (2012). ITS (JQ023163) and 28S nLSU (JQ023164) regions were sequenced, deposited in GenBank, and aligned with their closest BLAST matches from sequences deposited by Ferdman et al. (2005), Hansen et al. (2001, 2005), Trappe et al. (2010), and others.

Taxonomy

Carbomyces emergens Gilkey, N. Amer. Fl., Ser. 2, 1: 27 (1954) FIGS 1–3, 11–13

ASCOMATA subglobose, ovoid to pyriform, 1.2–3 × 1.5–4.4 cm, scattered or occasionally in small groups. PERIDIUM coriaceous, two-layered: outer layer very thin, membranous, initially whitish but finally yellow-brownish to dirty greyish, firmly attached, cracking by fissures or polygonal plates; inner layer ochraceous to dark brown, 0.2–0.6 mm thick. GLEBA multilocular, although apparently seeming to be solid, dark brown with sterile, lighter veins forming a fragile reticule; spore mass pulverulent, dark with olivaceous or vinaceous tints. ASCI globose to subglobose or ovoid, 8-spored, 40–55 × 30–5 µm, with thin brownish walls disintegrating at maturity. ASCOSPORES hyaline to light yellowish, globose, inamyloid, 9–14 µm diam., having a thick wall (1.2–1.5 µm) finely punctate when fully mature (as seen at 400× under LM, feebly verrucose under the SEM), but appearing smooth when immature.

SPECIMENS EXAMINED: MEXICO, CHIHUAHUA: Municipality of Juárez, in arid land: 20.IV.2005 R. Silva (UACJ 06); km 30 road Ciudad Juárez to Casas Grandes, next to *Larrea tridentata* Coville, 3.VIII.2008 M. Lizárraga, H. Pelayo (UACJ 1166 in AH 39344; GenBank Acc. Nos.—ITS: JQ023163, LSU: JQ023164); 31°23'20.21"N 106°23'32.94"W, alt. 1312 m, 27.III.2010, M. Lizárraga, M. Vargas, D. López (UACJ 1555); 8.V.2010 M. Lizárraga, D. López, F. Félix, M. Vargas (UACJ 1556); 15.IX.2010 (UACJ 1684); 6.XI.2010 M. Vargas, M. Lizárraga (UACJ 1687); 31°22'47.44"N 106°24'3.89"W, alt. 1319 m, 22.X.2010 (UACJ 1688); 30.X.2010 (UACJ 1700); 6.XI.2010 (UACJ 1689); 31°20'13.47"N 106°23'32.95"W, alt. 1334 m, 15.V.2010 M. Vargas, F. Félix, M. Lizárraga, D. López (UACJ 1686); 15.IX.2010 M. Vargas, M. Lizárraga (UACJ 1685); 10.IV.2011 (UACJ 1683); 31°19'39.98"N 106°30'7.43"W, alt. 1280 m, 23.V.2010 M. Lizárraga, D.

López, M. Vargas (UACJ 1557); 2.X.2010 M. Lizárraga, M. Vargas, D. Sáenz (UACJ 1628). **Municipality of Ojinaga**, La Mula, 29°14'24.0"N 104°26'09.0"W, alt. 1380 m, 24.V.2009 M. Lizárraga, C. Salazar, D. Sáenz, D. López, A. Gutierrez, E. Hernández (HM 22 in CESUES 7550); 24.X.2009 M. Lizárraga, A. Sánchez, A. Gutierrez, C. Salazar, D. López (HM 171 in CESUES 7551).

REMARKS— *Carbomyces emergens* has been repeatedly described (e.g., Trappe & Weber 2001) as having smooth or minutely roughened ascospores, but our studies show they are clearly warted at or above 400×. This may be because fully mature ascomata had not been found until now. The species could be macroscopically confused with *C. longii* and *C. gilbertsonii*, which share a similar habitat, but the species are easily differentiated by shape and spore ornamentation. Small sporocarps of *C. emergens* could easily be misidentified in situ as species of the basidiomycete genus *Arachnion* Schwein. (*Agaricaceae*) but in *Arachnion* the gleba lacks ascal remnants and contains a capillitium, and small (< 6 µm diam.) verrucose spores.

Zak & Whitford (1986) described the hypogeous development of *Carbomyces emergens* and reported the ascomata's apparent ingestion by rodents after collecting a few dried sporocarps in Northern Chihuahuan desert. Despite firm evidence, they suggested that fruiting probably occurs during late winter (January–March). Our collections UACJ 06 and UACJ 1557 are assumed to be immature, while the remaining specimens show an evident mature development.

Trappe & Weber (2001) regard *C. emergens* as the most common and widely distributed *Carbomyces* species, although reported only from American Southwest —Arizona, California, and New Mexico. Here we report it for the first time in Mexico as an apparently common species of the central Chihuahuan desert.

Carbomyces gilbertsonii N.S. Weber & Trappe, Harvard Pap. Bot. 6: 212 (2001)

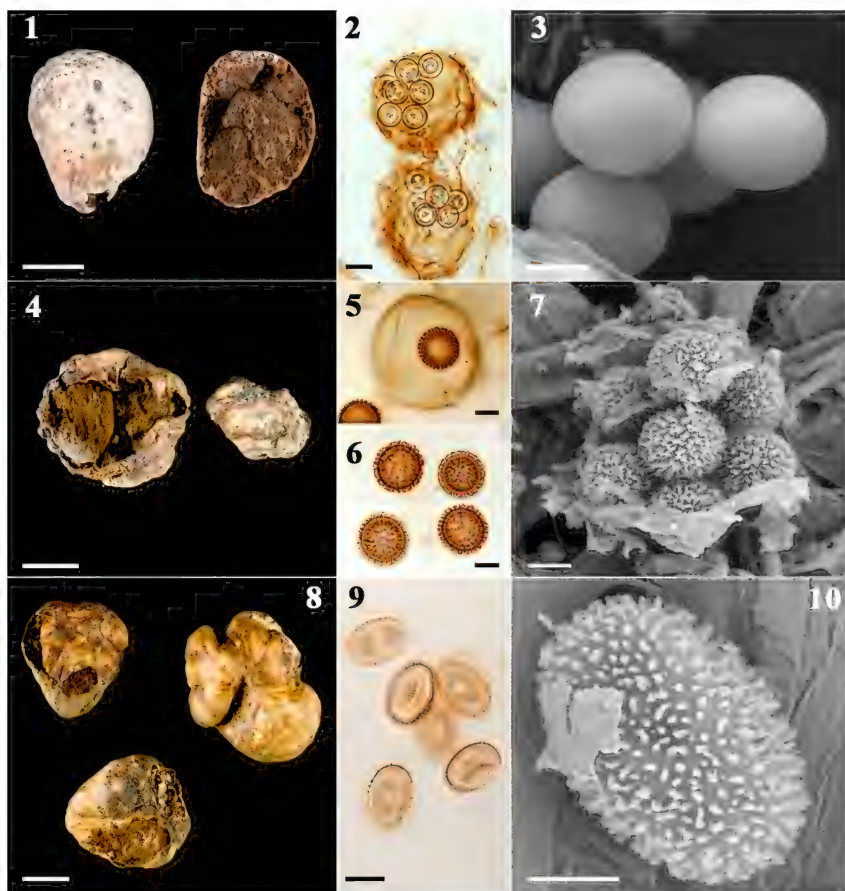
[nom. nov.]

FIGS 4–7, 14–16

= *Abstoma longii* Zeller, Mycologia 36: 628 (1944) [non *Carbomyces longii* Gilkey]

ASCOMATA immersed and finally superficial, globose to subglobose, laterally compressed to irregular, 2–3 cm diam. PERIDIUM two-layered, mostly persistent, ferruginous at maturity. GLEBA solid, pulverulent, brown to dark brown. ASCI subglobose, 8-spored, 40–65 µm diam., with thin light brown walls disintegrating at maturity. ASCOSPORES hyaline to brownish with age, globose to subglobose, inamyloid, 20–25 µm, ornamented with spine-like elements up to 2 µm long (LM). Under SEM such “spines” consist in small clumps of slender apically fused baculae, forming pyramids with hollow bases.

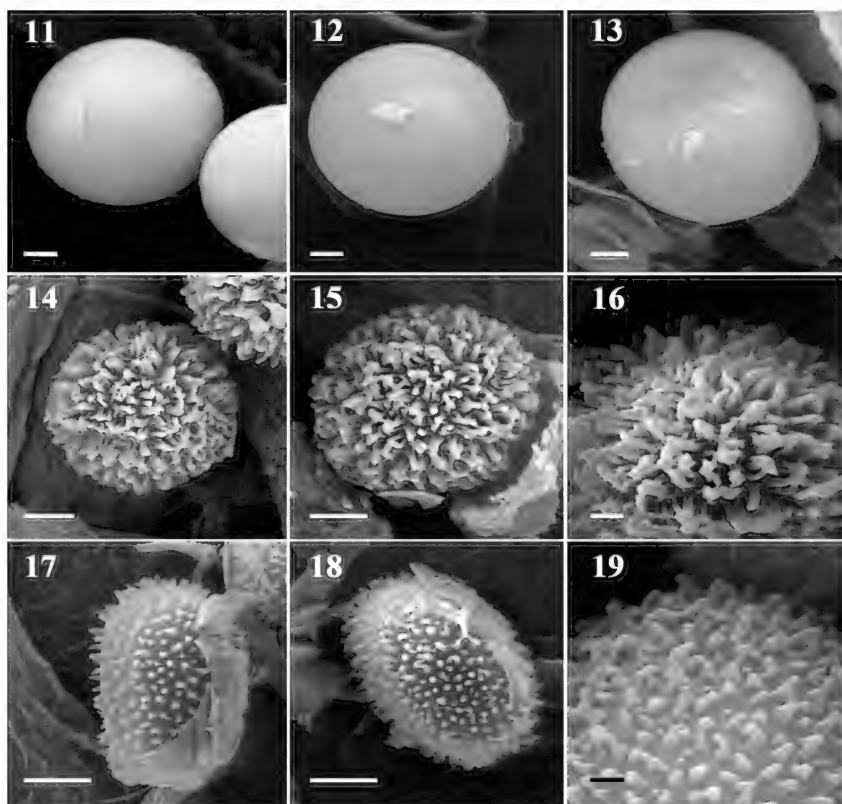
SPECIMENS EXAMINED: MEXICO, CHIHUAHUA: **Municipality of Juárez**, 31°22'47.44"N 106°24'3.89"W, alt. 1319 m, in arid soil near to *Larrea tridentata*, *Prosopis* sp. and *Opuntia* sp., 23.X.2010 M. Lizárraga, M. Vargas, (UACJ 1559, duplicate in AH 39346);



FIGS. 1–3: *Carbomyces emergens* AH 39344: 1. Ascocarps. 2. Asci with spores. 3. Spores (SEM). FIGS. 4–7 *Carbomyces gilbertsonii* AH 39347: 4. Ascocarps. 5. Asci with spores. 6. Free spores. 7. Spores (SEM). FIGS. 8–10 *Carbomyces longii* AH 39345: 8. Ascocarps. 9. Free spores. 10. Spore (SEM). Scale bars: 1, 4, 8 = 1 cm; 2, 5, 6, 9 = 10 μ m; 3, 10 = 5 μ m; 7 = 10 μ m.

6.XI.2010, (UACJ 1561 in AH 39347); (UACJ 1691); 31°23'20.21"N 106°23'32.94"W, alt. 1312 m, (UACJ 1690); 31°22'21.46"N 106°33'59.26"W, alt. 1225 m, 26.III.2011 (UACJ 1689).

REMARKS— *Carbomyces gilbertsonii* could be macro- and microscopically confused with *C. longii*, but small, ellipsoid spores (17–19 $\times$ 12–13 μ m) distinguish the latter species. Old *Scleroderma cepa* specimens could be confused with *C. gilbertsonii* specimens without ascus remains, but the smaller (9–12 μ m) spores of *S. cepa* spores are diagnostic. It is noteworthy that



FIGS. 11–13: *Carbomyces emergens* AH 39344: spores (SEM). FIGS. 14–16 *Carbomyces gilbertsonii* AH 39347: 14–15. Spores (SEM); 16. Spore ornamentation (detail, SEM). FIGS. 17–19 *Carbomyces longii* AH 39345: 17–18. Spores under SEM. 19. Spore ornamentation (detail, SEM). Scale bar: 11–13, 16 = 2 μ m; 14–15, 17–18 = 5 μ m; 19 = 1 μ m.

C. gilbertsonii was previously known only from the type locality (New Mexico, U.S.A.); our collections add Chihuahua (Mexico) to its distribution.

Carbomyces longii Gilkey, N. Amer. Fl., Ser. 2, 1: 27 (1954) FIGS 8–10, 17–19

ASCOMATA subglobose, laterally compressed, pyriform to irregular, 2–3 cm diam., 2.5–3 $\times$ 1.5–2 cm. PERIDIUM two-layered, persistent, outer layer white to greyish, later light brown to dark brown with reddish tinges at maturity. GLEBA pulverulent, dark brown to brown, becoming olivaceous. ASCI evanescent, only a few fragments of brownish, disintegrated walls were seen. ASCOSPORES light yellowish, ellipsoid, 17–19 $\times$ 12–13 μ m, inamyloid, ornamented with regularly

distributed spines $\leq 1 \mu\text{m}$ long (LM). Under SEM, the spines are scattered, usually with bent apices.

SPECIMENSEXAMINED: MEXICO, CHIHUAHUA: Municipality of Ahumada, 31°07'32.0"N 106°29'48.0"W, alt. 1280 m, in soil next to *Larrea tridentata*, 24.VII.2009 M. Lizárraga, C. Salazar, D. Sáenz, D. López, A. Gutierrez, E. Hernández (UACJ 1699). Municipality of Juárez, 31°23'20.21"N 106°23'32.94"W, alt. 1312 m, 27.III.2010 M. Lizárraga, M. Vargas, D. López (UACJ 1557 in AH 39017); 3.V.2010 (UACJ 1698); 15.V.2010 M. Lizárraga, D. López, M. Vargas, F. Félix (UACJ 1558); 15.IX.2010 M. Lizárraga, M. Vargas (UACJ 1697); 22.X.2010 (UACJ 1696); 6.XI.2010 (UACJ 1694); 31°22'47.44"N 106°24'3.89"W, alt. 1319 m, 30.X.2010 (UACJ 1562, duplicate in AH 39345); 6.XI.2010 (UACJ 1693); 31°22'21.46"N 106°33'59.26"W, alt. 1225 m, 26.III.2011 (UACJ 1695).

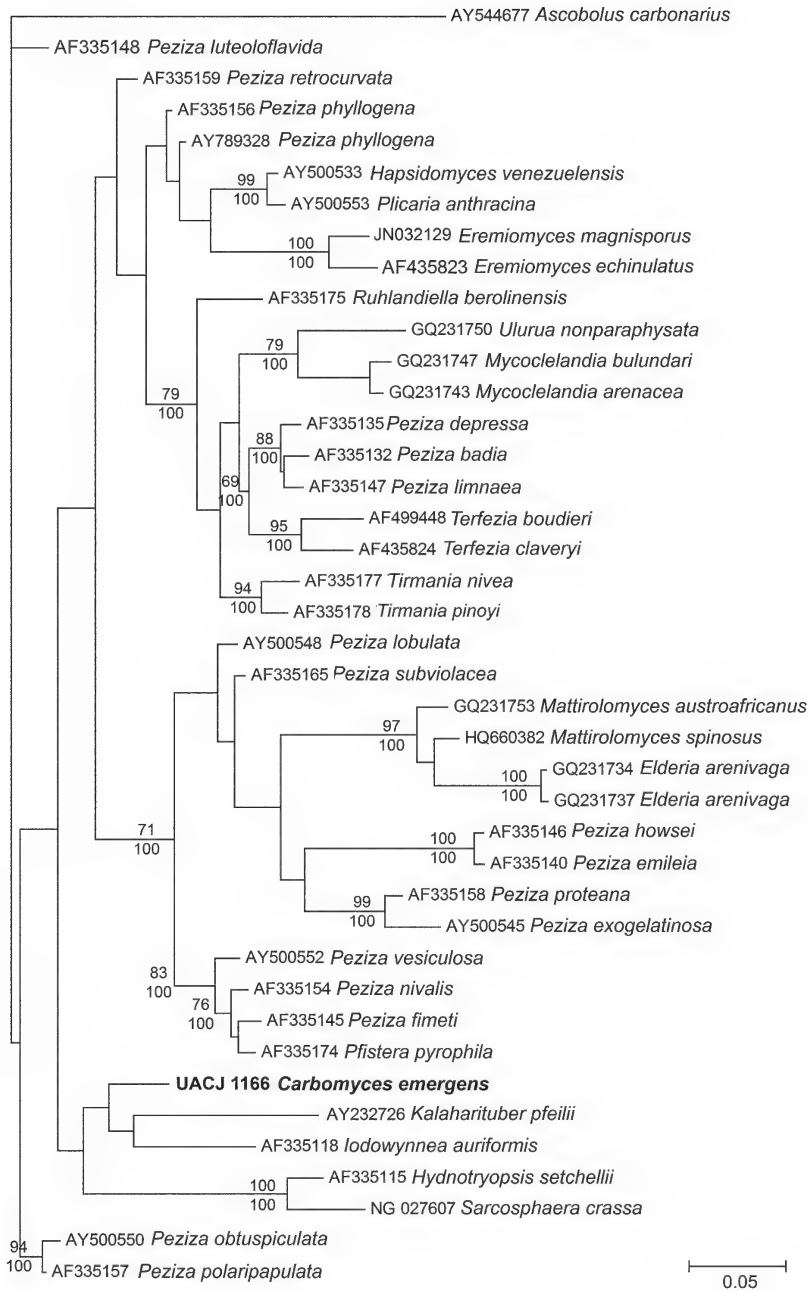
REMARKS— Since *C. longii* asci are fully evanescent, mature sporocarps could be confused with *Arachniopsis albicans* Long (*Agaricaceae*), but the latter is distinguished by small ($3\text{--}5 \times 3\text{--}4 \mu\text{m}$) oval feebly verrucose basidiospores and a hyaline capillitium. Co-occurring with *C. gilbertsonii*, *C. longii* was previously known only from the type locality (New Mexico, U.S.A.). Our report from Chihuahua, Mexico, represents the second world record.

Key to *Carbomyces* species in the Chihuahuan desert, Mexico

- 1 Spores globose and smooth to subsmooth (LM) or feebly warted (SEM) *C. emergens*
- 1' Spores spiny 2
- 2 Spores ellipsoid, scattered spines *C. longii*
- 2' Spores globose to subglobose, spines in small clumps forming pyramids
..... *C. gilbertsonii*

Molecular results

28S nLSU alignment comprised 39 sequences with 215 variable sites among 563 bases, 133 of them parsimony-informative. Bayesian and parsimony analyses were generally consistent with each other (Fig. 20) and supported the three main groups already recognized in Læssøe & Hansen (2007). The first group comprises *Kalaharituber*, *Iodowynnea*, *Hydnotryopsis*, *Sarcosphaera*, and several *Peziza* spp. The second group includes *Mattirolomyces*, *Elderia*, and a different set of *Peziza* spp. The third group includes *Terfezia*, *Tirmania*, *Mycoclelandia*, *Ulurua*, *Eremiomyces*, *Ruhlandiella*, *Hapsidomyces*, *Plicaria*, and a third set of *Peziza* spp. The analyses show *Carbomyces* as somewhat related to *Kalaharituber* (*Pezizaceae*) in the first group (Fig. 20), partially confirmed by BLAST of the only ITS sequence obtained, which linked *C. emergens* to *Terfezia* and *Kalaharituber* (84% and 83% identity in 68% and 65% coverage, respectively), in accordance with data obtained by Karen Hansen (pers. comm.).



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FIG. 20: 28S nLSU consensus phylogram constructed in MrBayes 3.1 showing the most probable relationships between *Carbomyces emergens* and its closest relatives in the *Pezizaceae*. Numbers close to nodes represent maximum parsimony bootstrap proportions (upper), and Bayesian posterior probabilities (lower). Only nodes supported by at least one inference method were annotated.

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***Cantharellus zangii*, a new subalpine basidiomycete from southwestern China**XIAO-FEI TIAN^{1, 2, 3}, BART BUYCK⁴, SHI-CHENG SHAO^{1, 2, 3},
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ABSTRACT — *Cantharellus zangii* is described and illustrated from subalpine forest (>3000 m) in northwestern Yunnan, China. The diagnostic characteristics of *C. zangii* are the small thin-fleshed fruit bodies with a long tapering fistulose stipe, a bright orange veined hymenophore, thin-walled clamped hyphae, and large ellipsoid basidiospores. So far it is only known from the type locality and its nearby area.

KEY WORDS — taxonomy, endemic species, ectomycorrhizal, *Cantharellales*

Introduction

Cantharellus Adans. ex Fr., the type genus of *Cantharellaceae*, was established by Fries (1821) and later typified with *C. cibarius* Fr. by Earle (1905). Thirteen species of *Cantharellus* have previously been reported from China: *C. appalachiensis* R.H. Petersen, *C. buccinalis* Mont., *C. cibarius*, *C. cinereus* (Pers.) Fr., *C. cinnabarinus* (Schwein.) Schwein., *C. friesii* Quél., *C. lateritius* (Berk.) Singer, *C. minor* Peck, *C. patouillardii* Sacc., *C. subalbidus* A.H. Sm. & Morse, *C. tuberculosporus* M. Zang, *C. vaginatus* S.C. Shao et al., and *C. yunnanensis* W.F. Chiu (Teng 1963, Chiou 1973, Zang 1980, Zhuang 2001, 2005, Tian et al. 2009, Shao et al. 2011). However, some of these collections are probably misidentified and need to be verified microscopically and molecularly,

as has been done by Buyck et al. (2011) and Buyck & Hofstetter (2011) for North America. During our *Cantharellus* research, we came across one small species with a very slender stipe in the subalpine mixed forest of Shangri-La (northwestern Yunnan, China), which we determined as distinct from already described species in the genus. It is here described as *Cantharellus zangii*.

Materials & methods

Macro-morphological fruitbody features were recorded and photographed in the field; color designations and codes follow Kornerup & Wanscher (1961). In basidiospore measurements, $[n/m/p] = n$ basidiospores measured from m basidiomata of p collections in 5% KOH solution, with dimensions given as $(a-)b-c(-d)$, where a and d are extremes and $b-c$ represent the interval range of 90% measured values. Q = basidiospore length/width ratio; Q_m = average Q of all basidiospores $\pm$ standard deviation. Herbarium abbreviation HKAS = Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences.

Taxonomy

Cantharellus zangii X.F. Tian, P.G. Liu & Buyck, sp. nov.

PLATE 1–5

MYCOBANK MB 563725

Differs from *Craterellus tubaeformis* by a plano-convex pileus and bright orange hymenium and from *Cantharellus queletii* in the fistulose stipe and hymenium with branching veins.

TYPE: China, northwestern Yunnan, Shangri La, Bitahai National Natural Reserve, 9.IX.2008, X.F. Tian 417 (Holotype, HKAS55791).

ETYMOLOGY: *zangii* is in honor of Professor Zang Mu (28 Dec. 1930–10 Nov. 2011), a distinguished Chinese mycologist who founded the Cryptogamic Herbarium in Kunming Institute of Botany, Academia Sinica (KUN-HKAS), and pioneered the study of cryptogam diversity in southwestern China.

MACROCHARACTERS — **BASIDIOMATA** small to medium, slender, submembranaceous, campanuliform to infundibuliform. **PILEUS** 2–3 cm in diam, at first umbonate then plane or slightly concave; squamules in the middle; ochre to ochre yellow (4B8–4B6), with tiny cinereous to grey (4B8–4B6); margin hygrophanous, smooth, with streaks, sometimes undulate, incurved at first, then straight. **STIPE** 7–8 $\times$ 0.3–0.4 cm, cylindrical to laterally compressed, sometimes slightly curved, attenuate towards base; entirely fistulose; dark orange-yellow (4B5–4B6) to bright orange (4A6). **LAMELLAE** decurrent, golden; 1.5–2 mm high, 1–2 mm apart, with transverse venations, with short branched lamellulae near the edge, seldom anastomosing. **CONTEXT** about 0.2 cm thick in the cap center; dull white (1A2), fibrous. **ODOR** *Osmanthus*-like. **TASTE** mild.

MICROCHARACTERS — **BASIDIOSPORES** $[85/4/2]$ $(8-)8.5-11 \times (4.5-)5-6.5$ (-7) μm , $Q = (1.3-)1.4-2(-2.2)$, $Q_m = 1.7 \pm 0.2$, ellipsoid or adaxially slightly

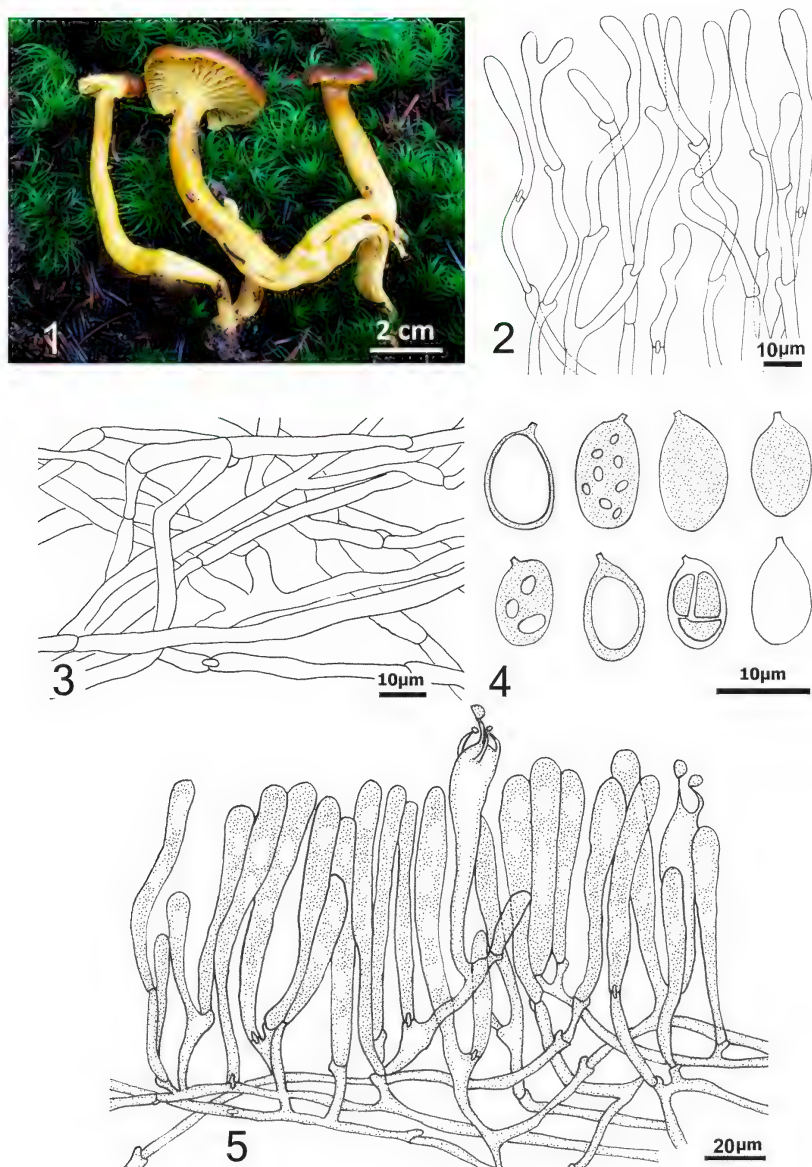


FIGURE 1–5. *Cantharellus zangii* (HKAS 55791, holotype): 1. Fruit bodies. 2. Hyphal extremities at the cap surface. 3. Tramal hyphae with clamps. 4. Basidiospores, often with many tiny oil drops. 5. Hymenium (with lengthening basidioles and basidia).

depressed and more or less reniform; thin-walled, hyaline, nearly colorless, sometimes with tiny oil drops. BASIDIA 75–85 × 6–9 µm, slender, clavate, 5(–6)-spored; sterigmata 5–6 µm long. CYSTIDIA absent. LAMELLAR TRAMA irregular, composed of colorless and loose hyphae, 3–5 µm diam. PILEIPELLIS a layer composed of mostly radially arranged hyphae, slightly brownish, 7–8 µm in diam; the terminal cell more or less clavate; CLAMPS very distinct and abundant in all parts.

ECOLOGY & DISTRIBUTION — Single or in groups on the ground in subalpine (>3000m) mixed forests dominated by *Larix potaninii* var. *macrocarpa* Y.W. Law and *Picea likiangensis* (Franch.) E. Pritz. So far known only from Shangri-La subalpine area, northwestern Yunnan, China.

ADDITIONAL SPECIMENS EXAMINED — CHINA. NORTHWESTERN YUNNAN, Shangri-La: Big Ravine, alt. 3030 m, 16.IX.2007, Y.C. Li 1537 (HKAS 55743); Haba Snowy Mountains, alt. 3000 m, 30.IX.2007, Feng Bang 182 (HKAS 55824).

Discussion

Based on its almost membranous pileus and veins as well as the hollow stipe, *Cantharellus zangii* could be taken for *Craterellus tubaeformis* (Fr.) Quél. (Redhead et al. 2002) at first glimpse. However, *C. zangii* often has an umbrella-shaped slender fruit body with a centrally plane or protruding pileus, whereas *Cr. tubaeformis* is horn-shaped with centrally depressed caps and has a grey or grey-white hymenium, never bright orange like *C. zangii*.

The membranous cap and slender stipe of *Cantharellus zangii* are also similar to the European *C. queletii* (Ferry) Corner (Corner 1966), which differs in a somewhat solid stipe and the absence of branching veins in the hymenium.

Cantharellus zangii also has some striking similarities with a few of the smaller American species (Petersen & Ryvarden 1971; Feibelman et al. 1996; Buyck et al. 2010). The yellow *C. minor*, for instance, shares the exceptional character of the fistulose stipe and thin-walled, voluminous hyphae in the pileipellis. However, its overall size is smaller, it also has slightly smaller spores, and the terminal cells in the pileipellis usually narrow at the extreme tips. The yellowish brown *C. tabernensis* Feib. & Cibula is similar in size to *C. zangii* with a very similar pileipellis, but the stipe is not hollow and the spores are much smaller. The same is true for *C. appalachiensis*, a more brownish and slightly more robust sister-species of *C. tabernensis*, possessing an identical microscopy.

The small size and abundant clamps together with the thin-walled hyphal extremities in the cap place this species in *C.* subgenus *Parvocantharellus* as defined by Eyssartier & Buyck (2001), where it is further diagnosed by its large spores and clavate terminal cells in the pileipellis.

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Seven lichen species new to Poland

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ABSTRACT — *Biatora pontica*, *Buellia violaceofusca*, *Catillaria croatica*, *Parmelia ernstiae*, *Placopsis lambii*, *Protoparmelia oleagina* and *Scoliciosporum curvatum* are recorded as new to Poland. Most reported species represent a group of crustose, often sterile lichens where secondary chemistry plays an important role in the taxonomy. Characteristics of each species, notes on similar taxa, distribution, and habitat are provided.

KEY WORDS — lichenized fungi, chemotaxonomy, lichen metabolites

Introduction

Since the publication of the list of lichens and lichenicolous fungi of Poland (Fałtynowicz 2003), numerous species have been added to the lichen biota of the country, including some new to science (e.g. Flakus 2007, Kukwa & Kubiak 2007, Motiejūnaitė & Czyżewska 2008, Krzewicka 2009, Kukwa & Flakus 2009, Łubek 2009, Zhurbenko et al. 2009, Śliwa & Flakus 2011). Most of these taxa are either lichenicolous fungi or lichens belonging to critical groups requiring analyses of secondary lichen metabolites.

In this paper, we report seven lichen species not previously reported from Poland.

Material & methods

The material studied is deposited in BILAS, KTC, OLS, PRA, UGDA, UPS, and herb. Printzen. Lichen substances were studied by thin-layer chromatography (TLC) using the methods of Culberson & Kristinsson (1970) and Orange et al. (2001). The examined localities are presented according to the modified ATPOL grid square system (Cieśliński & Fałtynowicz 1993, Kukwa et al. 2002).

The species

Biatora pontica Printzen & Tønsberg, Biblioth. Lichenol. 86: 140. 2003.

CHARACTERISTICS — The species is characterized by its thin, crustose and areolate thallus with punctiform to confluent, yellowish or light green soralia; areoles in non-sorediate parts reaching ≤ 1.2 mm in diam. Apothecia are flat to moderately convex, dark greyish-ochre to brownish-grey and 0.5–0.7 mm in diam.; sometimes they are immersed between soralia. Anatomically apothecia are characterized by the presence of a blue pigment (Pontica-blue) in the hypothecium, subhymenium, and (rarely) hymenium and epihymenium; sometimes an additional red pigment (Pontica-red) is produced in the hypothecium and hymenium. Ascospores are ellipsoid to bacilliform, $11.9\text{--}14.8 \times 3.4\text{--}3.7$ μm . *Biatora pontica* produces thiophanic acid (major), asemone, and an unidentified diagnostic substance called ‘pontica unknown,’ sometimes with minor or trace amounts of other xanthonenes; soralia react C+ orange (Printzen & Tønsberg 2003). In all Polish specimens thiophanic acid, asemone, and ‘pontica unknown’ were detected. In only one specimen were apothecia developed, but the fertile material is only the second such record in Europe (Printzen & Tønsberg 2003).

ECOLOGY & DISTRIBUTION — Previously *B. pontica* has been known from scattered localities in Europe (Austria, Italy, Norway, Russia, Slovenia), Asia (Turkey), and North America (USA) (Printzen & Tønsberg 2003, Santesson et al. 2004, Urbanavichus 2010). It is an epiphytic lichen growing in forests on deciduous trees in Europe and in Turkey, but in eastern North America it is more commonly found on *Picea orientalis* (Printzen & Tønsberg 2003).

In Poland *B. pontica* has been recorded in three localities in northern Poland, being always found on the bark of *Carpinus betulus* and *Fagus sylvatica* in the bottom of shady river or streams valleys.

SPECIMENS EXAMINED — **POLAND. DRAWSKIE LAKELAND.** Drawieński National Park, Stara Węgornia range in valley of the Płociczna river, c. 2 km NNE Ostrowite village, $53^{\circ}04'42\text{--}46''\text{N}$ $15^{\circ}59'31\text{--}41''\text{E}$, ATPOL grid square Cb–22, on *Carpinus betulus*, 08.05.2010, M. Kukwa 7948 (UGDA); **ELBLĄSKA UPLAND.** Valley of Srebrny Potok stream, forest section No. 342, $54^{\circ}10'28''\text{N}$ $19^{\circ}27'55''\text{E}$, ATPOL grid square Bd–06, beech forest in the bottom of the valley, on *Fagus sylvatica*, 05.08.2007, R. Szymczyk 641 (OLS L-691, dupl. in herb. Printzen); valley of Grabianka river, forest section No. 183b, $54^{\circ}16'42''\text{N}$ $19^{\circ}31'27''\text{E}$, ATPOL grid square Ad–96, beech forest, bottom of the river valley, on *Fagus sylvatica*, 27.06.2009, R. Szymczyk 657 (OLS L-762, dupl. in herb. Printzen).

COMMENTS — When fertile, *B. pontica* can be confused with *B. britannica* Printzen et al. (sorediate), *B. hypophaea* Printzen & Tønsberg (esorediate), and *B. ocelliformis* (Nyl.) Arnold (esorediate) —three other members of the genus with grey apothecia. They can be easily separated chemically as they produce argopsin (thalli react P+ orange-red) (Printzen et al. 2001, Printzen

& Tønsberg 2003); in Poland only *B. ocelliformis* has been recorded so far (Fałtynowicz 2003). Sterile specimens of *B. pontica* are morphologically very similar to *B. efflorescens* (Hedl.) Räsänen, which, however, contains argopsin (Printzen et al. 2001). *Biatora chrysantha* (Zahlbr.) Printzen and *Mycobilimbia epixanthoides* (Nyl.) Vitik. et al. can also be confused with *B. pontica*, but they contain gyrophoric acid (*B. chrysantha*) or no lichen metabolites are produced (*M. epixanthoides*) (Tønsberg 1992, Printzen & Tønsberg 2003, Printzen & Otte 2005).

Biatora pontica can also be confused with *Pyrrhospora quernea* (Dicks.) Körb. which is morphologically very similar when sterile (greenish sorediate thallus). Both taxa contain thiophanic acid as the major secondary metabolite, but *P. quernea* lacks the characteristic substance 'pontica unknown' (Tønsberg 1992, Printzen & Tønsberg 2003).

Buellia violaceofusca G. Thor & Muhr, Lichenologist 23: 11. 1991.

CHARACTERISTICS — The species is characterized by its thin, usually endophloeodal, pale grey or almost white thallus producing dark brownish soralia with a violet tinge (but greenish when abraded). Soralia are maculiform, scattered, slightly elevated and usually confluent; no apothecia have been discovered so far. This lichen does not produce secondary metabolites (Thor & Muhr 1991), but traces of terpenoids from tree bark can be detected by TLC.

ECOLOGY & DISTRIBUTION — *Buellia violaceofusca* is widespread but with a scattered distribution (Coppins et al. 2009); so far it has been found only in Europe in Austria (Poelt 1994), Belgium (Sérusiaux et al. 1999), Estonia (Thor & Nordin 1998), Great Britain (Coppins et al. 2009), Norway (Gaarder & Tønsberg 2010), Slovakia (Palice et al. 2006), and Sweden (Thor & Muhr 1991). The species usually grows on bark of *Quercus* spp. in different types of forest, but in Great Britain it has been reported also on *Fraxinus excelsior* (Coppins et al. 2009).

In Poland it has been found only at one locality in bark crevices of old *Quercus* sp. in a deciduous forest. Most probably the species is much more common in the country but has been overlooked due to the inconspicuous thalli and the habit of growing in deep crevices.

SPECIMEN EXAMINED — **POLAND. KASZUBSKIE LAKE LAND.** Trójmiejski Landscape Park, Dolina Ewy valley, 54°24'35"N 18°30'20"E, ATPOL grid square Ac-89, oak-hornbeam forest, on *Quercus* sp., 04.04.2004, M. Kukwa 3006 (UGDA L-10664).

REFERENCE MATERIAL EXAMINED — **SWEDEN. VÄRMLAND.** VISNUM KIL PAR., Nötön nature reserve, Arskagen, near Lake Vänern, 59°04'N 14°01'E, 50 m, on the northern side of old *Quercus robur* in deciduous wood, 27.05.1985, L.-E. Muhr 7911 (UPS L-17315 – holotype).

COMMENTS — *Buellia violaceofusca* is a very characteristic epiphytic species due to its thin thallus, violet tinged soredia, and the absence of lichen metabolites.

In Poland it can be confused with the morphologically similar *B. griseovirens* (Turner & Borrer ex Sm.) Almb. Both species have dark outermost soredia, but *B. griseovirens* has a thicker thallus and produces atranorin and norstictic acid, with trace of connorstictic acid and sometimes unidentified pigments (in some specimens only atranorin or only norstictic acid are present) (Thor & Muhr 1991, Tønsberg 1992, Coppins et al. 2009).

Buellia arborea Coppins & Tønsberg also develops pigmented soredia, but it can be readily separated by the presence of atranorin and placodiolic acid (Tønsberg 1992, Coppins et al. 2009); so far the species has not been reported from Poland, but its discovery there is probable.

Catillaria croatica Zahlbr., Ann. Mycol. 4: 487. 1906.

= *Lecania croatica* (Zahlbr.). Kotlov, Novosti Sist. Nizsh. Rast. 37: 251. 2004.

CHARACTERISTICS — As the only modern description of *C. croatica* (by Harris & Lendemer 2010) is based mainly on North American specimens, a more detailed description is provided below. The thallus of this corticolous lichen is light green-grey, superficial, well or poorly developed to immersed, consisting of scattered or almost contiguous areoles. Soralia are numerous, rounded, flat, convex or weakly capitate, discrete or patchily coalescing to form a leprose crust. Soredia are in shades of green, but sometimes externally pale brown pigmented. Apothecia are sessile, flat or weakly convex, with pale orange-cream or medium brown discs and paler margins. Anatomically, all structures are colourless except the pale brownish hypothecium. Asci are 8-spored with narrowly fusiform, 0–1-septate ascospores measuring 12–17 × 4.5 µm. Pycnidia were not found in Polish material. The species does not produce lichen substances detectable by TLC.

ECOLOGY & DISTRIBUTION — Rarely reported from Europe: Austria, Belgium, Croatia, France, Luxembourg, Romania, Slovakia, Slovenia and Ukraine (Printzen 1995, Mrak et al. 2004, Hafellner et al. 2005, Eichler et al. 2010, Vondrák et al. 2010), and also known from North America (Harris & Lendemer 2010). The species grows in forests, usually on bark of broad-leaved trees, rarely also shrubs (e.g. *Sambucus* sp.).

In Poland, *C. croatica* has been found in only one locality, a well-preserved, humid deciduous forest not seriously influenced by forest management, growing in association with *Anisomeridium polypori* (Ellis & Everh.) M.E. Barr, *Biatora ocelliformis*, *Lepraria lobificans* Nyl., *L. vouauxii* (Hue) R.C. Harris, *Opegrapha rufescens* Pers., *Pertusaria pertusa* (L.) Tuck., and *Bacidia* sp.

SPECIMEN EXAMINED — **POLAND, BIELSK PLAIN.** Białowiecki National Park, forest section No. 273, 52°43'32"N, 23°53'27"E, ATPOL grid square Cg-46, on *Acer* sp., 27.06.2009, A. Łubek s.n. (KTC).

COMMENTS — *Catillaria croatica* is an inconspicuous, easily overlooked, corticolous lichen with a sorediate thallus lacking lichen metabolites, with small

pale apothecia and 0–1-septate ascospores. The species is morphologically very similar to *Mycobilimbia epixanthoides*: both are sorediate, lack lichen substances, and produce pale biatorine apothecia. When fertile, the material is much easier to determine as both species differ in the type of spores: *C. croatica* produces 0–1-septate ascospores, whereas *M. epixanthoides* has 3-septate ascospores. In the sterile state they may be separated based on substrate preference: *Catillaria croatica* grows exclusively on the bark of trees, and *M. epixanthoides* prefers bryophytes and is rarely corticolous (Hafellner et al. 2005, Harris & Lendemer 2010). The corticolous material of the latter, according to the cited sources, could be distinguished by irregular young soralia forming larger irregular patches, while soralia of *C. croatica* remain discrete and round, even when they become crowded with age. From our experience, this might not be true in all cases and some sterile samples may remain undetermined.

Several other sorediate species, which are morphologically similar to *C. croatica* (e.g. *Biatora efflorescens* or *B. chrysantha*), can be confused with this lichen, but they are all readily distinguished by their secondary metabolites (e.g., Printzen 1995).

Catillaria croatica has been recently transferred to *Lecania* A. Massal. by Kotlov (2004), but molecular studies have shown that it nests in *Bilimbia* De Not. s.l. and is not phylogenetically related to *Lecania* s. str. (Reese Næsborg et al. 2007). Therefore we prefer to keep the species in the admittedly heterogeneous genus *Catillaria* A. Massal.

Parmelia ernstiae Feuerer & A. Thell, Mitt. Inst. Allg. Bot. Hamburg 30–32: 52. 2002.

CHARACTERISTICS — This foliose lichen is characterized by its heavily pruinose thalli and isidia, small and non-overlapping lobes, commonly present lobulae, and the production of atranorin (minor amount, together with chloratranorin), salazinic acid (major amount, with minor amount of consalazinic acid), lobaric, lichesterinic, protolichesterinic, nephrosteranic, isonephrosterinic (all minor amounts) and protocetraric (trace amount) acids (Feuerer & Thell 2002, Thell et al. 2008). Although not distinctly lobulate, Polish material agrees well with the description, except nephrosteranic, isonephrosterinic and protocetraric acids were not detected, most probably due to their low quantity or overlapping with other metabolites in a similar position on the TLC plates.

ECOLOGY & DISTRIBUTION — *Parmelia ernstiae* is widely distributed in Europe, with a few records known from Africa. It has been reported from Austria, Belgium, Bosnia and Herzegovina, Britain, Bulgaria, Czech Republic, Denmark, Estonia, France, Germany, Greece, Ireland, Lithuania, Luxembourg, the Netherlands, Slovenia and Spain (Feuerer & Thell 2002, Sérusiaux et al. 2003, Thell 2003, Molina et al. 2004, Otte 2005, Santesson et al. 2004, Thell

et al. 2007, Suija et al. 2007, Motiejūnaitė et al. 2008, Thell et al. 2008, Berger et al. 2009, Seaward 2010, Diederich et al. 2011) and in Africa from Algeria and the Canary Islands (Sérusiaux et al. 2003). It is a typically corticolous species growing on deciduous or rarely coniferous trees in open situations (e.g. roadsides, churchyards) and forests (Thell 2003, Santesson et al. 2004, Thell et al. 2007); an exceptional saxicolous record is also known (Thell et al. 2008).

In Poland it has been found only at one locality in northwestern Poland on a roadside elm in a well-lit situation. All available material labelled as *P. saxatilis* in UGDA has been examined, but as no more specimens of *P. ernstiae* have been traced, it probably should be regarded as rare in the country.

SPECIMEN EXAMINED — POLAND. DRAWSKIE LAKELAND. road N of Głusko village, 53°02'59"N 15°56'34"E, ATPOL grid square Cb-22, on roadside *Ulmus* sp., 05.05.2010, M. Kukwa 7793 (UGDA).

COMMENTS — Based on DNA sequences and different morphology (Feuerer & Thell 2002) and more recently on the composition of secondary metabolites (Thell et al. 2008), *P. ernstiae* has been recently segregated from *P. saxatilis* (L.) Ach., from which it can be easily separated morphologically by the epruinose thalli and isidia and chemically by the absence of lobaric and fatty acids (Feuerer & Thell 2002, Thell et al. 2008).

Parmelia ernstiae can also be confused with another isidiate species, *P. serrana* A. Crespo et al. Thalli of both taxa are pruinose, but in *P. serrana* lobes are larger, rounded, and commonly overlapping and lobaric acid is not produced (Molina et al. 2004, Thell et al. 2008). *Parmelia serrana* has not been found in Poland, although its distribution range (see Thell et al. 2008) suggests it probably occurs there.

Placopsis lambii Hertel & V. Wirth, Flecht. Baden-Württ. Verbreit.: 511. 1987.

CHARACTERISTICS — The species is characterized by its placodioid thallus with deeply incised and radiating marginal lobes, shiny upper surface, usually blackish and more or less rounded soralia, non-lobate cephalodia (absent in some specimens), and the production of 5-O-methylhiascic and gyrophoric acids as major substances (Moberg & Carlin 1996, Gilbert & Purvis 2009, Harrold et al. 2010). The cephalodia were not developed in Polish specimen.

ECOLOGY & DISTRIBUTION — *Placopsis lambii* is widespread but rather scattered. In Europe it has been reported from Austria, Belgium, Britain, Finland, France, Germany, Iceland, Ireland, the Netherlands, Norway, Russia, and Sweden (Wirth 1987, Diederich 1994, Moberg & Carlin 1996, Aptroot et al. 1999, Hafellner & Türk 2001, Santesson et al. 2004, Harrold et al. 2010, Seaward 2010, Diederich et al. 2011). In Africa it has been found in Kenya, Lesotho, and Tanzania (Moberg & Carlin 1999) and in the Americas in Bolivia, Chile, Costa Rica, and Ecuador (Galloway 2002, Galloway & Arvidsson 2007). It is also

known from New Zealand (Galloway 2001) and asiatic Russia (eastern Siberia and Russian Far East; Urbanavichus 2010).

In Poland *P. lambii* has been recorded only once in northern Poland where it was found on a pile of stones in a sunny but rather humid habitat near several ponds. This stand is also noteworthy for other rare and endangered lichens in Poland, e.g., *Rhizocarpon lecanorinum* Anders and *Xanthoparmelia mougeotii* (Schaer.) Hale.

SPECIMEN EXAMINED — POLAND. KASZUBSKIE LAKELAND. Vicinity of Szumleś village, 54°09'33"N 18°13'26"E, ATPOL grid square Bc-07, open area by small ponds, on stone, 08.05.2008, M. Kukwa 5968, M. Buliński, J. Zarembka (UGDA L-16515, dupl. in BILAS and PRA).

COMMENTS — *Placopsis lambii* is very similar and morphologically almost indistinguishable from *P. gelida* (L.) Linds. *Placopsis gelida* tends to be matte or shiny only at the lobe tips of the upper surface and has more elongated soralia that are rarely blackish. However, those are subjective characters, and the chemistry is a most reliable diagnostic feature; both taxa contain gyrophoric acid, but *P. lambii* additionally produces 5-O-methylhiascic acid (Gilbert & Purvis 2009, Harrold et al. 2010). Due to their similarity one could treat both species as one variable entity, but molecular studies supported their distinctiveness (Schmitt et al. 2003).

Some *P. lambii* morphs might be also mistaken for *Trapelia placodioides* Coppins & P. James, which, however, lacks radiating marginal lobes and cephalodia; *T. placodioides* also produces gyrophoric acid, but 5-O-methylhiascic acid is absent (Gilbert & Purvis 2009, Purvis et al. 2009).

Protoparmelia oleagina (Harm.) Coppins, Lichenologist 24: 368. 1992.

= *Lecanora oleagina* Harm., Lich. France 5: 1023. 1913.

CHARACTERISTICS — *Protoparmelia oleagina* is a crustose, often sterile, epiphytic lichen with a more or less continuous, dull olivaceous brown, scurfy granular-isidiate thallus and apothecial margin; the apothecial disc is also olivaceous. Ascospores are fusiform, $9.5\text{--}15 \times 2\text{--}3.5 \mu\text{m}$ (Coppins & Chambers 2009, Brodo & Aptroot 2005). The species produces lobaric acid, but often in low concentration (Brodo & Aptroot 2005). One Polish specimen was fertile (Kukwa 7837a), but very few apothecia were developed.

ECOLOGY & DISTRIBUTION — *Protoparmelia oleagina* occurs only in Europe, where it has been recorded from Austria, Belgium, Finland, France, Germany, Great Britain, Italy, the Netherlands, Norway and Sweden (Aptroot et al. 1999, Scholz 2000, Hafellner & Türk 2001, Santesson et al. 2004, Brodo & Aptroot 2005, Nimis & Martellos 2008, Coppins & Chambers 2009, Diederich et al. 2011).

In Poland *P. oleagina* has been found on deciduous trees in humid forests at two localities in western Pomerania (NE Poland).

SPECIMENS EXAMINED — **POLAND. DRAWSKIE LAKELAND.** Drawieński National Park, eastern slopes of Drawa river valley, 2 km NE of Zatom village, Tragankowe Urwisko range, 53°09'12"N 15°51'49–54"E ATPOL grid square Cb–12, deciduous forest, on *Fagus sylvatica*, 06.05.2010, M. Kukwa 7837a (UGDA). **SŁOWIŃSKIE COAST.** Białogóra nature reserve, forest section No. 22, 54°49'27"N, 17°57'52"E, ATPOL grid square Ac–36, wet pine-birch forest, on *Betula pendula*, 22.09.2010, M. Kukwa 8268a, A. Jabłońska, M. Oset (UGDA L-16660).

COMMENTS — *Protoparmelia oleagina* is easily distinguished by its corticolous habitat and more or less continuous, dull olivaceous brown, scurfy granular and isidiate thallus. Morphologically it is most similar to *P. ochrococca* (Nyl.) P.M. Jørg. et al., but the thallus of the latter is not isidiate, consists of subglobose areoles, and lacks lichen substances (Brodo & Aptroot 2005, Coppins & Chambers 2009); so far *P. ochrococca* has not been recorded from Poland.

Chemically and morphologically *P. oleagina* can be confused with *P. hypotremella* Herk et al., which is known from several localities in Poland (see Kubiak et al. 2010). Both taxa contain lobaric acid, but the thallus of *P. hypotremella* consists of dispersed round flat granules or microsquamules; it is also brighter, pale grayish-brown to olive-grey (Brodo & Aptroot 2005, Coppins & Chambers 2009).

Morphologically, *P. oleagina* resembles small forms of *P. badia* (Hoffm.) Hafellner, but the latter is a saxicolous lichen with ellipsoid-fusiform ascospores with distinctly pointed apices (Coppins & Chambers 2009).

Some epiphytic species of the genus *Lecanora* Ach. also have dark apothecial discs (e.g. *L. argentata* (Ach.) Malme or *L. persimilis* (Th. Fr.) Arnold), but they lack brown pigmentation, often produce atranorin or usnic acid in the cortex, and their ascospores are generally wider (Śliwa 2007, Coppins & Chambers 2009).

Scoliciosporum curvatum Sérus., Nord. J. Bot. 13: 458. 1993.

CHARACTERISTICS — This crustose lichen is characterized by its pale green to dark grey-green granular thallus, chlorococcoid photobiont with rather large cells (12–)14–20(–22) μm , numerous and very small (up to 0.16 mm diam.), pale pink, orange to brown apothecia, 2 μm wide, simple to furcated or rarely anastomosing paraphyses, 8–16-spored asci and 1-septate, curved (lunulate) or slightly sigmoid spores with acute ends, 7–11 $\times$ 1.5–3 μm . Paraphyses are abundant only in young apothecia. The species does not produce lichen substances (Sérusiaux 1993, Edwards et al. 2009).

ECOLOGY & DISTRIBUTION — This species occurs only in Europe, being much more common in western countries. It has been reported from Austria, Britain, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Norway, Slovakia, Romania, Spain, Sweden, Switzerland and Ukraine (Sérusiaux 1993, Poelt 1994, Boom et al 1995, Palice 1999, Söchting & Alstrup 2002, Clerc 2004,

Santesson et al. 2004, Lisická 2005, Edwards et al. 2009, Vondrák et al. 2009, Seaward 2010, Dymytrova 2011). Dymytrova (2011), citing Nimis & Martellos (2003), cited *S. curvatum* from Italy, but Nimis & Martellos (2008) have included it not in the Italian checklist but only in the iconographic archive (along with several other species not reported from Italy). The report by Himmelbrant (2008) of *S. curvatum* from Poland is a misprint (Himmelbrant, pers. comm.), making the specimens cited below the first records for Poland.

Most central European localities of *S. curvatum* lie in the mountains (490–1600 m a.s.l.), but those from Poland are in the lowlands. The species is treated as an endangered species (extremely rare and potentially endangered or vulnerable) in some countries and has been included in the lichen red lists of Austria (Türk & Hafellner 1999), Czech Republic (Liška et al. 2008), and Switzerland (Scheidegger et al. 2002).

Scoliciosporum curvatum is typically a foliicolous lichen growing in sheltered and humid situations on leaves and (sometimes) twigs of evergreen shrubs such as *Buxus*, *Camellia*, and *Rhododendron* (Edwards et al. 2009). The species has also been found on needles (rarely twigs) of spruces, firs and occasionally pines, typical of southwestern Scandinavia (Santesson et al. 2004) and the mountains of central Europe (Poelt 1994, Palice 1999, Guttová & Palice 1999, Lisická 2005).

In Poland *S. curvatum* has been found only in two localities in Romincka Forest in northeast Poland where it was collected from spruce needles inside a wet spruce forest and in a managed pine forest.

SPECIMENS EXAMINED — POLAND. ROMINCKA FOREST. Puszcza Romincka Forest Landscape Park, Żytkiejmska Struga nature reserve, forest section No. 63, c. 3.5 km WSW of Żytkiejmy village, 54°20'51"N 22°37'55"E, ATPOL grid square Af-86, boggy spruce forest, on needles of spruce, 17.09.2003, A. Zalewska, W. Fałtynowicz s.n. (OLS); forest section No. 184, c. 4.5 km of SW Żytkiejmy village, 54°19'49"N 22°37'37"E, ATPOL grid square Af-86, pine forest with spruce, on needles of spruce, 19.09.2003, A. Zalewska, W. Fałtynowicz s.n. (OLS).

COMMENTS — Placement of *S. curvatum* within *Scoliciosporum* A. Massal. is problematic as it is separated from other representatives by its higher number of ascospores per ascus and different organization of paraphyses (Sérusiaux 1989, 1993). The generic concept appears to be unclear, since several other species with deviating characters are included, especially *S. abietinum* T. Sprib., *S. intrusum* (Th. Fr.) Hafellner, and *S. coniectum* Kantvilas & Lumbsch (Sérusiaux 1993, Hafellner 2004, Spribille et al. 2009, Kantvilas & Lumbsch 2010). Molecular analyses would clarify its circumscription and explain its relationship with *S. curvatum*.

Scoliciosporum curvatum is inconspicuous and easily overlooked, as its thallus may resemble a green algal cover over the substrate. In the field it can be confused with other species growing in the same habitat, but it is

easily recognizable after microscopic examination. In Poland, only two other species, *Fellhanera bouteillei* (Desm.) Vězda and *F. subtilis* (Vězda) Diederich & Sérus., have been found on needles of *Abies alba* (*F. bouteillei*) or *Picea abies* (*F. subtilis*) (Fałtynowicz 2003). The thallus of *F. subtilis* is grey to green with smaller photobiont cells (5–15 µm in diam.), apothecia are whitish and larger (0.15–0.4 mm in diam.), ascospores are oblong, fusiform and (1–)3-septate, and it always develops pinkish pycnidia. *Fellhanera bouteillei* has a verruculose-rimose thallus, often entirely covered by dull bluish-green farinose soredia. The apothecia and pycnidia superficially resemble those of *F. subtilis*, but ascospores are only 1-septate (as in *S. curvatum*) but also ovoid to oblong-ovoid (sometimes soleiform) and often constricted at the septa; the *F. bouteillei* thallus also contains usnic acid, zeorin, and $\pm$ asemone (Sérusiaux 1996, Aptroot et al. 2009).

Some *S. curvatum* specimens might also be mistaken for *Fellhanera viridisorediata* Aptroot et al., which has not been found in Poland. It has a similar green granular thallus, with firstly punctiform, then coalescing, soralia and (0–)1-septate elongate-ellipsoid ascospores. The species is often sterile, but then it differs in the presence of roccellic acid (Aptroot et al. 2009).

Scoliciosporum gallurae Vězda & Poelt, recently recorded in Poland (Kukwa & Kubiak 2007), which may also superficially resemble *S. curvatum*, produces gyrophoric acid and is known from twigs in open, nutrient-enriched situations.

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***Dissoconium proteae* newly recorded from China**

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ABSTRACT — We document the first report of *Dissoconium proteae* from China. This fungus was found on the cuticle of apple fruit collected from orchards in Shaanxi Province. Its morphology is compared with other *Dissoconium* species and a phylogenetic analysis based on ITS sequence is presented.

KEY WORDS — sooty blotch and flyspeck, taxonomy, internal transcribed spacer, rDNA

Introduction

Sooty blotch and flyspeck (SBFS) is a worldwide disease complex more than 60 fungi that blemish the cuticle of pome fruits (such as apple) in humid temperate regions and cause cosmetic damage entailing significant economic losses (Batzner et al. 2005). The common name “flyspeck” refers to species in the SBFS complex that appear on fruit surfaces as clusters of black, shiny, round to oval, flattened sclerotium-like bodies with no mycelial mat (Batzner et al. 2005).

The hyphomycete genus *Dissoconium* de Hoog et al. has lightly pigmented conidiophores that produce septate and aseptate conidia in a sympodial manner. The conidia are hyaline, one- or two-celled, and detach forcibly in pairs (Hoog et al. 1983).

Currently the genus includes nine species: *Dissoconium aciculare* de Hoog et al., *D. australiense* Crous & Summerell, *D. commune* Crous & Mansilla, *D. dekkeri* de Hoog & Hijwegen, *D. eucalypti* Crous & Carnegie, *D. mali* G.Y. Sun et al., *D. musae* Arzanlou & Crous, *D. proteae*, and *D. subuliphorum* (Matsush.) R.F. Castañeda (Arzanlou et al. 2008; Crous et al. 1999, 2004, 2007, 2008; Hoog et al. 1983, 1991; Zhang et al. 2007). Batzner et al. (2005) reported five putative *Dissoconium* species within the sooty blotch complex on apple fruit.

Recently, during investigations of SBFS of apple in China, we identified four fungal isolates that represent the first record of *Dissoconium proteae* from China based on ITS sequence analysis and morphological comparison.

Materials & methods

ISOLATION. In September 2008, apples with flyspeck signs were collected from orchards in Shaanxi Province. The thalli were transferred to a potato dextrose agar slant (potato 200 g, dextrose 20 g, agar 10 g; PDA) directly from the apple surface, and then cultured at 23±1°C in darkness (Sun et al. 2003). Colony description of the species was based on a 1-month-old pure culture. Morphological characteristics are described from specimens obtained from a cover slip inserted into the surface of the media. Voucher specimens and cultures are conserved in the Fungal Herbarium of Northwest A&F University, Yangling, China (HMUABO).

DNA SEQUENCING. Template DNA was extracted from fungal mycelium according to Li et al. (2011); primer pairs used for ITS amplification and sequencing were ITS1-F

TABLE 1. Sequences used in the phylogenetic analysis

SPECIES	GENBANK CODE	REFERENCE
<i>Dissoconium proteae</i> (mynbx10)	HM070373	This paper
<i>D. proteae</i> (mynbx12)	HM070374	This paper
<i>D. proteae</i> (mynbx1.1)	HM070375	This paper
<i>D. proteae</i> (mynxc1.1)	HM070376	This paper
<i>D. aciculare</i>	AY725519	Crous et al. (2004)
	AY725521	Crous et al. (2004)
<i>D. australiense</i>	EF394854	Crous et al. (2007)
<i>D. eucalypti</i>	EF394855	Crous et al. (2007)
<i>D. mali</i>	EF627451	Zhang et al. (2007)
<i>D. musae</i>	EU514226	Arzanlou et al. (2008)
	EU514225	Arzanlou et al. (2008)
<i>D. proteae</i>	EU707897	Crous et al. (2008)
<i>Dissoconium</i> sp.	AY598874	Batzer et al. (2005)
	AY598875	Batzer et al. (2005)
	AY598877	Batzer et al. (2005)
	FJ425205	Díaz Arias et al. (2010)
<i>Mycosphaerella communis</i> (anam. <i>D. commune</i>)	AY725544	Crous et al. (2004)
	DQ302950	Crous et al. (2006)
<i>M. lateralis</i> (anam.: <i>D. dekkeri</i>)	EU301078	Burgess et al. (2007)
<i>Ramichloridium apiculatum</i>	EU041794	Arzanlou et al. (2007)
	EU041791	Arzanlou et al. (2007)
<i>R. cerophilum</i>	EU041798	Arzanlou et al. (2007)
<i>R. indicum</i>	EU041799	Arzanlou et al. (2007)
<i>Ramichloridium</i> sp.	FJ425199	Díaz Arias et al. (2010)
<i>Stenella araguata</i>	AF362066	Crous et al. (2001)

(Gardes & Bruns 1993) and ITS4 (White et al. 1990). Amplification was completed with the following cycling parameters: initial denaturation at 94°C for 3 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, and extension at 72°C for 10 min. The PCR products were sequenced by Organism Technology Co., Shanghai, China.

The ITS nucleotide sequences generated in this study were added to sequences downloaded from GenBank (TABLE 1) that had high similarity according to a BLAST search (National Center for Biotechnology Information's nucleotide blast program). Preliminary alignments were performed using CLUSTAL-X (1.83) (Thompson et al. 1997), imported into BioEdit version 5.0.9.1 (Hall 1999), and manually adjusted. Phylogenetic analysis of aligned DNA sequences was performed with PAUP version 4.0b10 for 32-bit Microsoft Windows (Swofford 2001). Heuristic searches were performed with 1000 random sequence additions. Clade stability was evaluated by 1000 bootstrap replications. Other measures for parsimony, including tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively), were also calculated. *Stenella araguata* was used as the outgroup taxon.

Results: DNA phylogeny

A multiple alignment of the rDNA-ITS was generated with 21 sequences obtained from GenBank plus the sequences of isolates mynboxd10, mynboxd2, mynboxd1.1 and mynxc1.1. An MP tree with 354 length (CI = 0.7232, RI = 0.8622, RC = 0.6235) was constructed (Fig. 1). Two major clades were resolved in the MP trees. One of the major clade with 100% bootstrap value contained three species in *Dissoconium*, *Mycosphaerella*, and *Ramichloridium*. The other major clade also had a bootstrap value of 100%. Our isolates and an authentic *Dissoconium proteae* isolate identified by Crous et al. (2008) fell within a single clade with 71% bootstrap support.

Taxonomy

Dissoconium proteae Crous, Persoonia 20: 68. 2008.

FIG. 2

Mycelium external consisting of branched, septate, smooth, hyaline hyphae, 0.8–2.8 µm wide. Conidiophores 9–20 × 2.2–4.7 µm, solitary, arising from hyphae, subcylindrical, subulate, tapering to a bluntly rounded or truncate apex, straight to gently curved, smooth, hyaline, becoming medium pale brown with age, aseptate; conidiogenous loci terminal and lateral, visible as slightly thickened, darkened scars, 0.5 µm wide. Conidia 6.3–11.5 × 2.2–4 µm, solitary, hyaline to pale olivaceous, smooth, ellipsoid, non-constricted to slightly constricted at median septum, apex obtuse, base obconic-truncate, tapering pronounced at somewhat protruding hilum, unthickened, not darkened, 1 µm wide. Secondary conidia developing adjacent to primary conidia, hyaline to subhyaline, aseptate, ellipsoid, tapering prominently towards a protruding, truncate base, 4.3–8 × 1.6–3.8 µm; anastomosing with primary conidia after

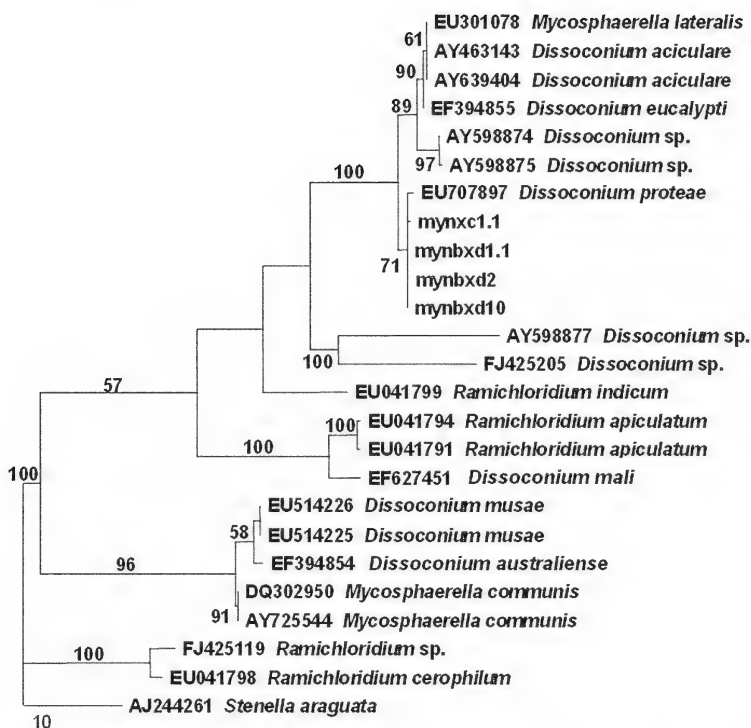


FIG. 1 The majority consensus tree (length = 354, CI = 0.7232, RI = 0.8622, RC = 0.6235) derived from a heuristic search option in PAUP version 4.0b10 for 32-bit Microsoft Windows with 1000 randomizations of sequence input orders and 1000 bootstrap replications using the data set of ITS1, 5.8S and ITS2. Bootstrap values higher than 50% are indicated above or below the tree branches.

active discharge. In some cases the secondary conidia were observed to germinate.

CULTURAL CHARACTERISTICS: Colonies on PDA spreading, with sparse aerial mycelium and regular smooth margins; surface white or sienna, with patches of white and cinnamon; forming clusters of black sclerotia (remaining infertile) on PDA; reaching 15 mm diam after 1 mo.

CHARACTERISTICS ON THE HOST: On apple peel, the fungus produced dark, shiny, round to oval, slightly protuberant sclerotium-like bodies (FIG. 2A).

SPECIMENS EXAMINED: China: Shaanxi Province, Xianyang, Bin County, 35°04'N 108°09'E, alt. 1108 m, on *Malus ×domestica* (Rosaceae), 18 Sep. 2008, Y.N. Mao, HMUABO (the Fungal Herbarium of Northwest A&F University) 8885, 8877, 8866 (with dried culture), culture mynxbd10, mynxbd2, mynxbd1.1. China: Shaanxi Province, Xianyang, Xunyi County, 35°13'N 108°33'E, alt. 1300 m, On *Malus ×domestica*, 18 Sep. 2008, Y.N. Mao, HMUABO 8682 (with dried culture), culture mynxc1.1.

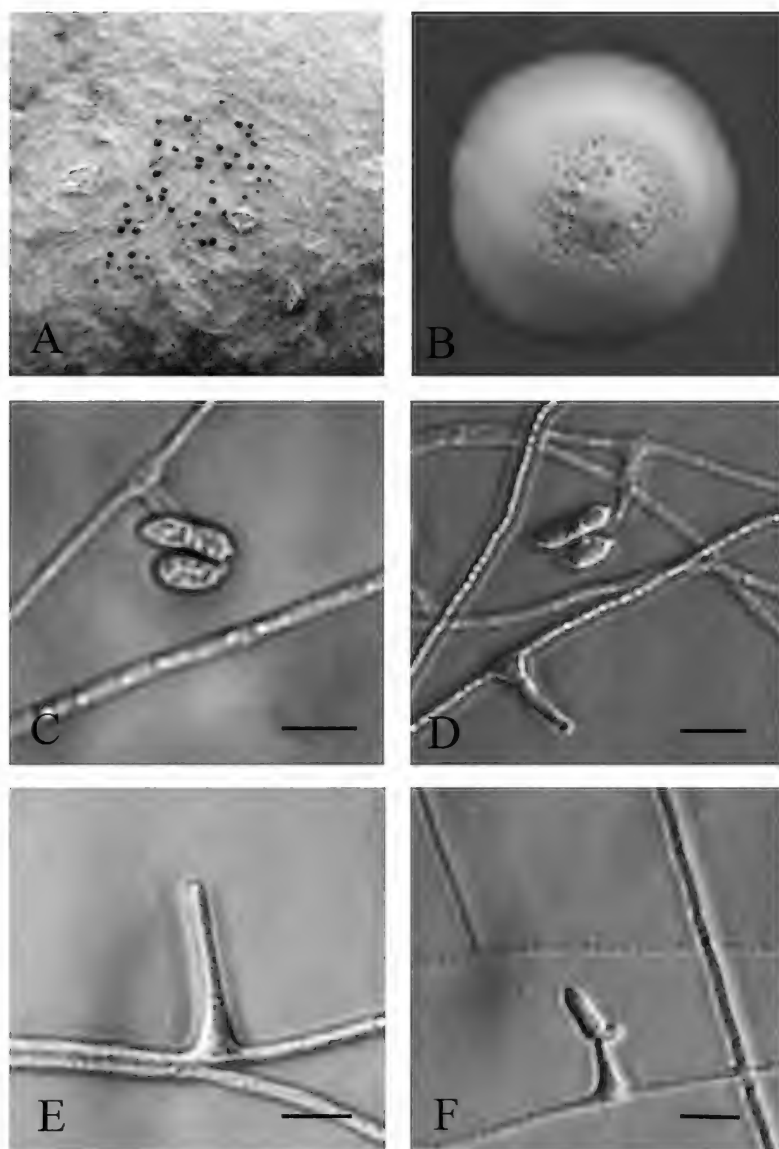


FIG. 2 *Dissoconium proteae* isolate mynboxd10. A. Signs on apple peel. B. Colony on PDA agar after 30 days. C-D. Conidia, conidiogenous cells, and hyphae. E. Conidiogenous cell. F. Conidium and secondary conidium. Bars: B=10 mm, C-F =10 μ m.

Discussion

Of the more than 60 fungal species that blemish the surface of apples worldwide (Díaz Arias et al. 2010), many have been described quite recently. For example, Li et al. (2010) and Ma et al. (2010) reported three species of *Zygophiala* (*Z. cryptogama* and *Z. cylindrica*; *Z. qianensis*) associated with flyspeck on apples. Our report provides the first evidence that *Dissoconium proteae* can cause flyspeck on apple fruit. Crous et al. (2008) described *D. proteae* from leaves of *Protea* sp. (*Proteaceae*), causing a leaf spot.

Currently there are nine species in *Dissoconium*. Based on phylogenetic analysis of the ITS region and morphological characters of the anamorph, we identified four isolates as *Dissoconium proteae*. This is the first report of the species from apple, and a new record for China.

Acknowledgments

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The fungal collection of the Jagiellonian University Herbarium (KRA), Kraków, Poland

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ABSTRACT — The paper presents a short history of the mycological collection of the Jagiellonian University Herbarium (KRA, Kraków, Poland). The Herbarium holds over 22,000 specimens, including some interesting European and extra-European fungal exsiccata. One of the most valuable fungal collections (including many type specimens) in the Herbarium is that containing Javanese fungi gathered by Marian Raciborski at the end of 19th century.

KEY WORDS — fungi of Pieniny Mts., fungi of Gorce Mts., ascomycetes, basidiomycetes, *Peronospora*

The history

The origin of the Jagiellonian University Herbarium dates back to 1780, when the Faculty of Chemistry and Natural History was established, and with it the Natural History Room (Köhler 1999; Köhler 2001a). The Herbarium, which was long associated with the Chair of Botany at the Botanical Garden, is now part of the Institute of Botany of Jagiellonian University.

The first record of fungi being kept in the Herbarium dates from 1812, and the oldest specimen with a date placed on the label is from 1807 (FIG. 1). In the past the Herbarium collections served mainly an educational function (Köhler 2001b). Until 1910 each set of specimens, including purchased, exchanged, or donated exsiccata and collections, was kept separately and a taxonomic criterion was not applied to the Herbarium's arrangement. Between 1910 and 1918 the collections were merged, and all specimens were arranged taxonomically (Köhler 2001a).

After the Second World War the fungal collection was separated from plant collections and arranged by Barbara Gumińska, who in 1950 became the first curator of the Herbarium's mycological section (Köhler 2001b) and served



PLATE 1. The oldest specimen in the fungal collection of the Jagiellonian University Herbarium: "*Geastrum hygrometricum* Pers. Normandie. 1807. D. Delaroche (?). Habitat in Italia, Helvetia, Germania umbrosis. 4."

as curator until her retirement in 1993. The next curator, Katarzyna Turnau (1993–2005), was succeeded by Piotr Mleczko, who currently holds this post.

Until 2006 the specimens in the mycological collection were not numbered. In that year all specimens were counted and most of them were assigned numbers. The collection now contains 20,195 numbered specimens: 5564 *Ascomycota*, 11,098 *Basidiomycota*, 2682 anamorphic fungi, and 851 fungus-like organisms (mostly *Peronospora* in the *Oomycota*). In addition it holds also about 900 unnumbered macromycete specimens collected by Barbara Gumińska in Pieniny National Park, Poland.

The collections

One of the most valuable fungal collections is the Javanese collection of Marian Raciborski. Raciborski's collection dates back to 1896–1900 and consists of dried specimens marked with 'J' (Java) after the number (FIG. 2) as well as some samples preserved in ethanol-formaldehyde solution, which are in the Botanical Garden Museum (Köhler & Zemanek 1989). According to Wojewoda (1986), Raciborski described 235 new fungal taxa from Java, all tropical microfungi except for one macromycete. Many genera and species described by Raciborski are still accepted under his names: e.g. *Aldona*

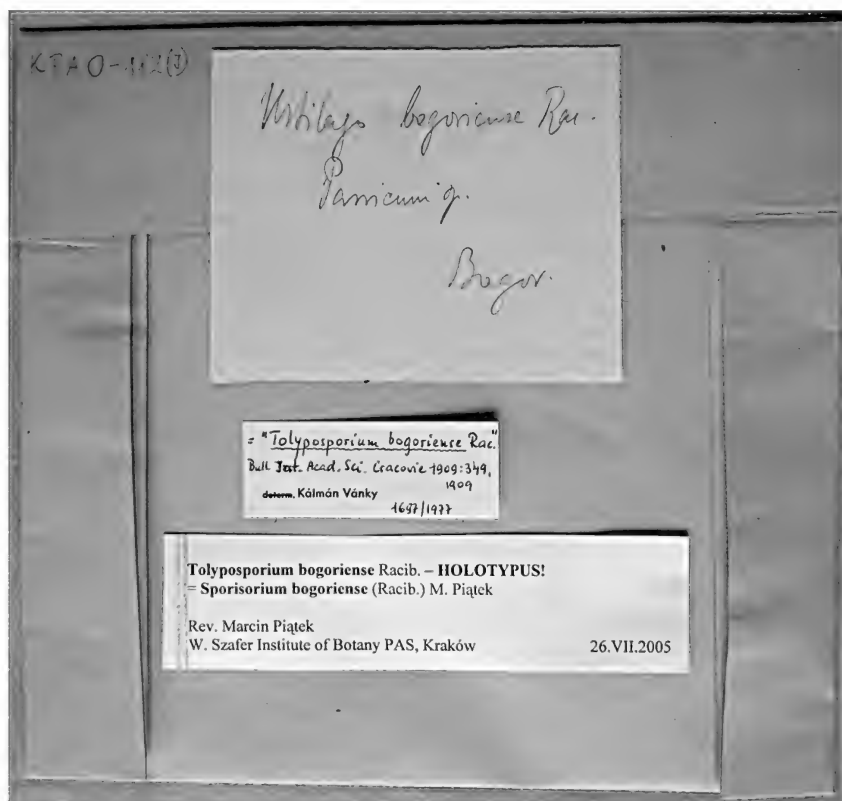


PLATE 2. A specimen from the Javanese collection of Marian Raciborski: "*Ustilago bogoriense* Rac., *Panicum* sp., Bogor."

(*A. stella-nigra*), *Alina* (*A. jasmini*), *Anhellia* (*A. tristis*), *Balladyna* (*B. gardeniae*), *Farysia* (*F. javanica*), *Goplana* (*G. aporosae*, *G. micheliae*, *G. mirabilis*), *Lambro* (*L. insignis*), *Ordonia* (*O. orthobasidion*), *Skierka* (*S. agallocha*, *S. canarii*), and *Telimena* (*T. erythrinae*) (Index Fungorum 2011).

Other important exsiccata kept in KRA are those of Karl Wilhelm Gottlieb Leopold Fuckel, Otto Jaap, Gottlob Ludwig Rabenhorst, Heinrich Rehm, Pier Andrea Saccardo, Trajan Săvulescu, Hans Sydow, and Charles Wright (see the list below), which comprise many syntypes. At present only the exsiccata of *Ascomycota* are catalogued, comprising 134 syntypes.

The fungal section also holds many specimens gathered in Poland, including three holotypes: *Exobasidium dubium* Racib., *Wawelia regia* Namysl. (an exotic species found in the Botanical Garden of the Jagiellonian University, Kraków), and *Geopyxis rehmi* Turnau. The most important Polish collections were

contributed by Władysław Wojewoda (1932–2010) from Ojców National Park (macromycetes), Katarzyna Turnau from the Gorce Mts. (larger ascomycetes), and Barbara Gumińska from Pieniny National Park. Recently Piotr Mleczko and co-workers made a significant collection of fungi, mostly from southern Poland, which is now being incorporated into the Herbarium and will increase the number of specimens by approximately 1000.

The most important fungal exsiccata and collections in the Herbarium mycological section are listed below. It should be noted that the Taxonomic Literature series (Stafleu & Cowan 1976–88; Stafleu & Mennega 1992–2000) and the monographs by Stevenson (1971) and Pfister (1985) mostly do not mention the sets stored at Jagiellonian University Herbarium.

The names of fungal collections, dates, and authors follow (where possible) Stafleu & Cowan (1976–88), Stafleu & Mennega (1992–2000), Pfister (1985), or Stevenson (1971). Otherwise data are based on exsiccata labels or, with respect to author life spans, from various sources cited with each collection).

Abbreviations

IF – Index Fungorum (www.indexfungorum.org)

SC-1–7 – Stafleu & Cowan (1976–88), vols. 1–7

Pf – Pfister (1985)

St – Stevenson (1971)

POLISH COLLECTIONS

- Fungi bialowiezenses exsiccati, Wincenty Siemaszko (1887–1943) [Köhler 2002, Pf, SC-5].
- Fungi parasitici Poloniae exsiccati, Marian Raciborski (1863–1917) [Piekietko-Zemanek 1986, Wojewoda 1986].
- Mycotheca polonica, Marian Raciborski and Bolesław Namysłowski (1882–1929) [Köhler 2002, Wojewoda 1986].
- Mycotheca polonica, Józef Kochman (1903–95) and Bogusław Sałata (1940–99) [Majewski 1995, Chmiel & Mułenko 1999, Pf].

EUROPEAN COLLECTIONS (EXCEPT POLAND)

- Ascomyceten, Ascomycetes exsiccatae, Heinrich Rehm (1828–1916) [St; Pf; SC-6].
- Cryptogamae Cechoslovenicae exsiccatae, František Šmarda (1904–68) [SC-5].
- Flora Hungarica, Gabriel Bohus (1914–2005) [IF].
- Flora hungarica exsiccata. Sectio botanica Musei Nationalis Hungarici, Budapest.
- Flora Lituana Exsiccata Fungi parasitici, Antanas Minkevičius (1900–98) [Botanikos ir genetikos katedra 2011; Pf].
- Flora Romaniae exsiccata, Alexandru Borza (1887–1971) [Pop 1972; Pf; SC-1].
- Fungi bavarici exsiccati, Andreas Allescher (1828–1903) and Johann Nepomuk Schnabl (1853–99) [Pf; SC-1,5].
- Fungi bohemici, František Bubák (1865–1925) [SC-1].

- Fungi Europaei exsiccati Klotzschii herbarii vivi mycologici continuatio. Editio nova. Series secunda, Gottlob Ludwig Rabenhorst (1806–81) [Pf; St; SC-4].
- Fungi rhenani exsiccati, Karl Wilhelm Gottlieb Leopold Fuckel (1821–76) [SC-1].
- Fungi Schemnitzensis, Andreas Kmet (1841–1908) [Lizoń 1973; Pf].
- Fungi selecti exsiccati, Otto Jaap (1864–1922), Carl Brick (1863–1924) [Pf; SC-2].
- Griby Rossii, Gavril Stepanowich Nevodovskij (1874–1952) and Voldemar Tranzschel (1868–1942) [Karczmarz & Sałata 1982; Pf; SC-6].
- Herbarium mycologicum romanicum, Trajan Săvulescu (1889–1963) [Pf; SC-5].
- Klotzschii herbarium vivum mycologicum, Gottlob Ludwig Rabenhorst [Pf; St; SC-4].
- Klotzschii herbarium vivum mycologicum, editio novo, Gottlob Ludwig Rabenhorst [Pf; St; SC-4].
- Kryptogamae exsiccatae editae a Museo Palatino Vindobonensi, Günter von Beck (1856–1931), Alexander Zahlbruckner (1860–1938), Karl von Keissler (1872–1965), Franz Petrak (1886–1973) [Pf; St; SC-1,2,7].
- Mycotheca germanica, Paul Sydow (1851–1925), Hans Sydow (1879–1946) [Pf; SC-6].
- Mycotheca veneta, Pier Andrea Saccardo (1845–1920) [Pf; SC-4].
- Plantae cryptogamicae, quas in Arduenna collegit (Plantae cryptogamicae Arduennae), Marie-Anne Libert (1782–1865) [Pf; SC-3].

EXTRA-EUROPEAN COLLECTIONS

- Cryptogamae parasiticae in Insula Javae lectae exsiccatae, Marian Raciborski [Pf]
- Fungi cubenses wrightiani, Charles Wright (1811–86) [St; SC-7].
- Fungi exotici exsiccati, Hans Sydow [St; Pf; SC-6].
- Ohio Fungi, William Ashbrook Kellerman (1850–1908) [St; Pf; SC-2].

Loan requests should be directed to: Institute of Botany Herbarium (Fungal collection), Jagiellonian University, Kopernika 31, 31-501 Kraków, Poland.
Curator of the mycological collection: Dr. Piotr Mleczko.

The Herbarium web-page is <http://www.ib.uj.edu.pl/en/?d=zielnik>

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***Mycena pseudoinclinata*, new to Italy**

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ABSTRACT – The first report of *Mycena pseudoinclinata* in Italy is accompanied by notes on the taxonomy, ecology, and distribution of this uncommon basidiomycete.

KEY WORDS – *Mycenaceae*, sect. *Fragilipedes*, basidiomycetes

Introduction

Mycena pseudoinclinata (*Mycenaceae* Overeem) belongs to sect. *Fragilipedes* (Fr.) Quél., which is considered the largest section in *Mycena* (Pers.) Roussel. The species was originally described by Smith (1947), who distinguished it from *M. inclinata* (Fr.) Quél. by the presence of both pleurocystidia and cheilocystidia and absence of (or fewer) fibrillose flecks on the stipe. This taxon has a limited distribution all over the world, particularly in Europe where it has recently been recorded only in Switzerland and France (Robich 2003). This paper deals with the first record of *M. pseudoinclinata* in Italy, which we recently collected in a reforested area in the neighborhood of the town of Palermo (Sicily).

Materials & methods

Basidiomata were identified while fresh and microscopic features were observed in H₂O using a Leica DMLB microscope; spore measurements were based on 100 observations. Nomenclature follows the Index Fungorum (<http://www.indexfungorum.org/Names/Names.asp>). The description of the characteristics of the species is based on personal observations that were subsequently compared with the descriptions given by Smith (1947) and Robich (2003). The collection is stored in the fungal dried reference collection of the Herbarium Mediterraneum Panormitanum, Palermo (PAL).

Taxonomy

Mycena pseudoinclinata A.H. Sm., North Amer. Species *Mycena*: 327. 1947.

FIGS. 1B, 2

Pileus 20–30 mm, ash-gray, conical to campanulate, pruinose, surface moist and glabrous, disc slightly umbonate, margin striate. Lamellae close, white, notched, margin concolorous to the face of lamellae; lamellulae present. Stipe 70 × 3 mm, fragile, hollow, almost concolorous with the pileus, darker at the base with a slight whitish felt. Flesh typically with a farinaceous odor and taste.

Basidiospores 8–10 × 6–6.5 µm, ellipsoid to broadly ellipsoid, smooth, amyloid. Basidia, 30–35 × 7.5–8.5 µm, tetrasporic, claviform. Cheilocystidia 20–60 × 9–12 µm, claviform, sphaerical-pedicellate, with irregular excrescences of different shapes ≤ 10–15 µm in length, well-spaced. Epicutis composed of cylindrical hyphae, smooth, 2–5 µm diam., with terminal elements 50–70 × 2–6 µm. Subhymenial layer with sub-globose hyphae, isodiametric, 3–8 µm diam. Hyphae of stipe cylindrical, innermost 8–25 µm and outermost 1.5–2.5 µm diam., smooth or with sparse excrescences, dextrinoid.

COLLECTION EXAMINED: ITALY. SICILY: Raffo Rosso, neighborhood in Palermo, 423 m, on rotten trunks of *Eucalyptus camaldulensis* Dehnh. (*Myrtaceae*), 13 Jan 2011, GPS: 38°10'46"N 13°15'40"E, coll. R. Compagno & A. La Rosa (PAL 960).

COMMENTS — Robich (2003) included mycenas with large basidiomata in sect. *Mycena*. Some species that might be confused with others in sect. *Filipedes* (Fr.) Quél. or *Fragilipedes* can be differentiated by their basidioma shape and cheilocystidial type. Mycenas in sect. *Fragilipedes* are primarily differentiated through cheilocystidial and/or pleurocystidial shapes, here the main character differentiating *M. pseudoinclinata* is the numerous large irregular protuberances on the cheilocystidia. Although Smith (1947) referred to pleurocystidia in his original description of *M. pseudoinclinata*, Maas Geesteranus (1988a,b) correctly described this species as lacking them. Robich, who cited the cheilocystidia as restricted to the lamellar edge (Robich 2003), includes the species in his revised key to *Mycena* sect. *Fragilipedes* of the northern hemisphere.

Smith (1947) first reported *M. pseudoinclinata* as densely cespitose and occurring in Tennessee, Pennsylvania, and Michigan (U.S.A.). Bi et al. (1993) report the species from China on fallen twigs in broad-leaved woods. In Europe Gerault (2005) reports the species from France and Switzerland in broad-leaved forests mainly characterized by oaks.

We found that *M. pseudoinclinata* also grows in *Eucalyptus* plantations (FIG. 1A) and confirm its role in decaying wood and its typical cespitose habit, as demonstrated by our collection of eight connate basidiomata per trunk (FIG. 1B). The literature notes that *M. pseudoinclinata* fruits from spring (April) through autumn; in Sicily we have collected basidiomata as early as January, no

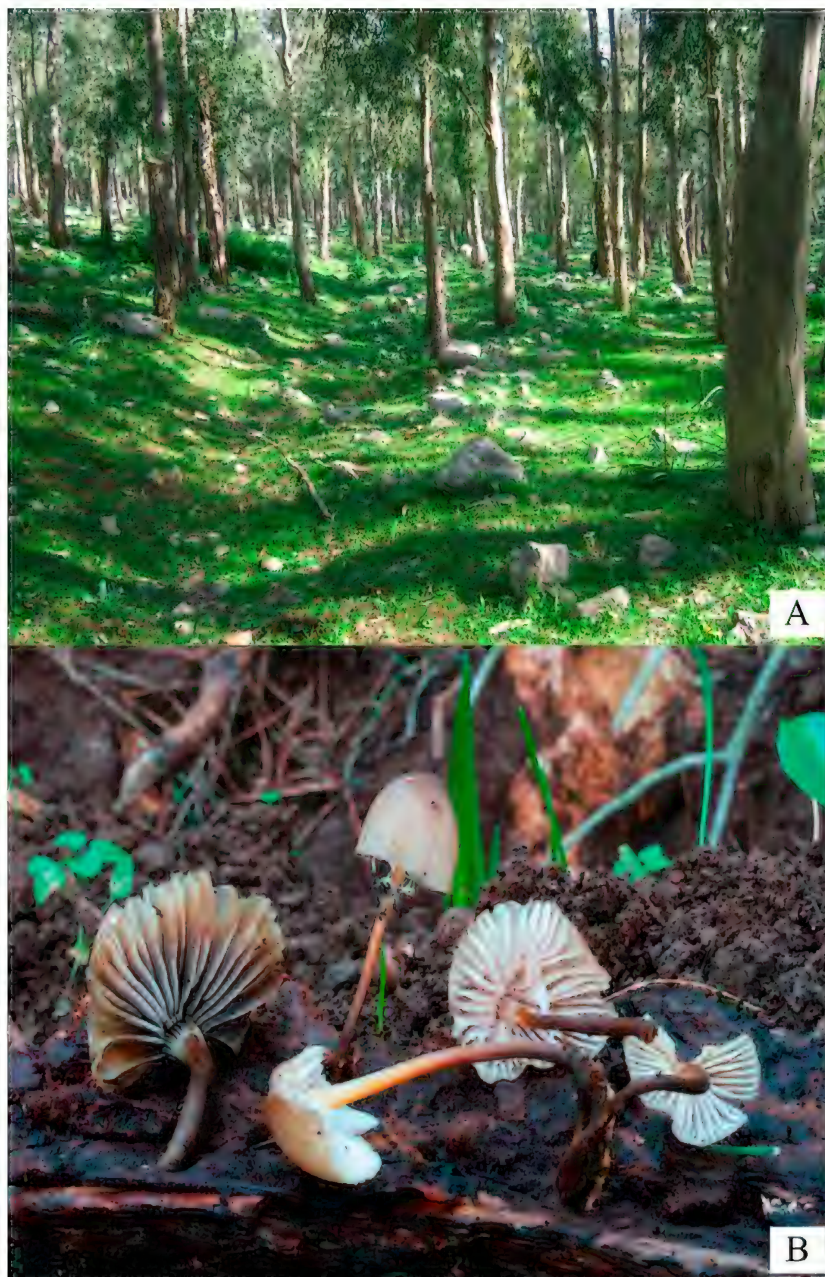


FIG. 1: *Mycena pseudoinclinata*.
A. Habitat in *Eucalyptus camaldulensis* plantation. B. Basidiomata.

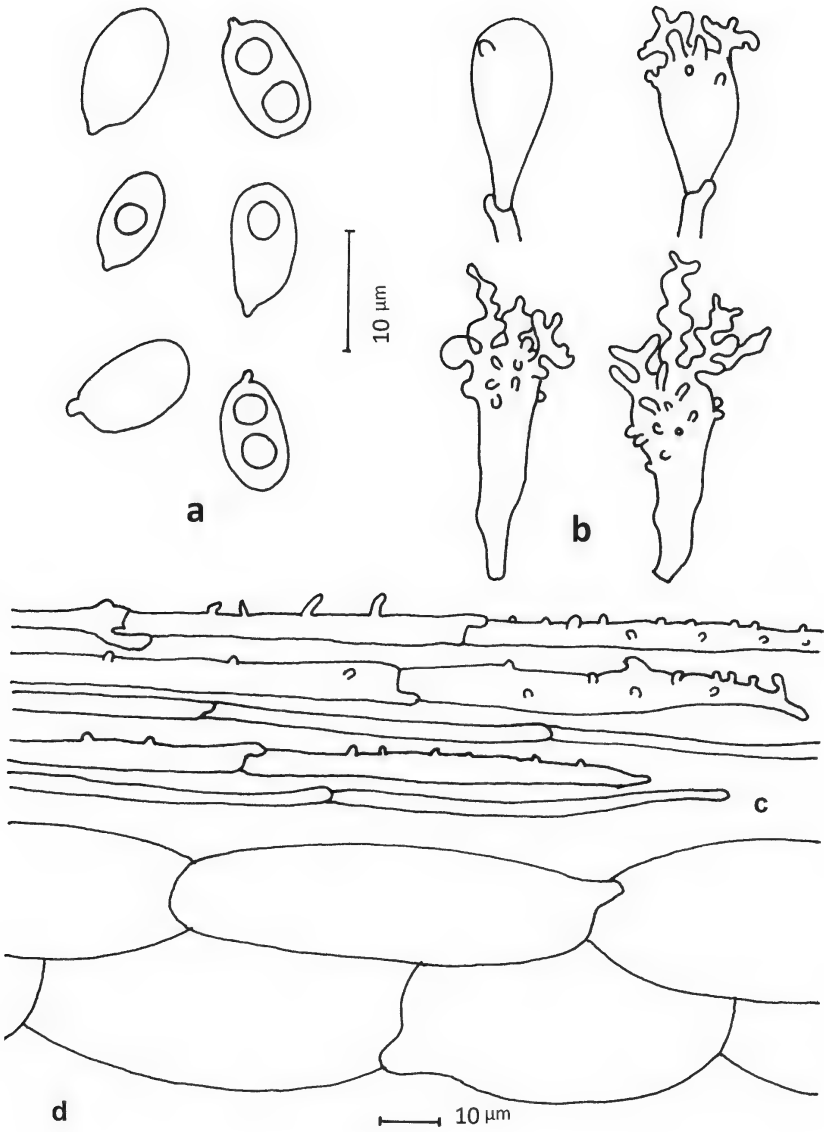


FIG. 2: *Mycena pseudoinclinata* (PAL 960(Mic)].
a. Basidiospores. b. Claviform cheilocystidia with irregular excrescences of different shapes.
c. Epicutis with cylindrical hyphae. d. Vesiculose and swollen hyphae in the pileitrama.

doubt due to the mild weather conditions on the island. Our report represents the southernmost limit of its distribution in Europe.

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***Rossbeevera yunnanensis* (Boletaceae, Boletales),
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ABSTRACT — A new sequestrate (truffle-like) species, *Rossbeevera yunnanensis*, is described based on a collection from Chuxiong Prefecture, Yunnan Province, China. The species is morphologically characterized by its large, narrow, fusoid to fusiform basidiospores and remarkably thin peridium. Maximum likelihood and neighbor joining phylogenies of our nLSU rDNA dataset indicate that the species constitutes the earliest diverging lineage within the genus *Rossbeevera* and has a close phylogenetic relationship to species of *Leccinellum*. Morphological and phylogenetic relationships to the other Asian members of *Rossbeevera* are discussed.

KEY WORDS — *Chamonixia*, FTA, hypogeous fungi, *Leccinum*, *Rosbeeva*

Introduction

The sequestrate (truffle-like) genus *Rossbeevera* T. Lebel & Orihara (erroneous orthographic variant: “*Rosbeeva*”) was erected to accommodate Australasian and Asian species of *Chamonixia* Rolland (Lebel et al. 2012a,b), which are phylogenetically close to but distinct from *Chamonixia* sensu stricto within *Boletaceae*. The genus is characterized by ellipsoid to fusiform basidiospores with 3–5 longitudinal ridges, bluish green to cyanescent discoloration of basidiomata, and a thin cutis-like whitish peridium. Phylogenetically, this genus is closely related to the epigeous bolete genera *Leccinum* Gray and *Leccinellum* Bresinsky & Manfr. Binder and sequestrate genera *Chamonixia* and *Octaviania* Vittad. (Lebel et al. 2012a, Orihara et al. 2012). *Rossbeevera* species have

been previously reported from Australia—*R. vittatispora* (G.W. Beaton et al.) T. Lebel and *R. westraliensis* T. Lebel, New Zealand—*R. pachydermis* (Zeller & C.W. Dodge) T. Lebel, Southeast Asia—*R. mucosa* (Petri) T. Lebel, China—*R. bispora* (B.C. Zhang & Y.N. Yu) T. Lebel & Orihara, and Japan—*R. eucyanea* Orihara and *R. griseovelutina* Orihara (Lebel et al. 2012a,b).

During a fieldtrip in Yunnan Province, China, two of the authors (Smith & Ge) collected *Rossbeevera* basidiomata that could not be ascribed to any currently known species. Here we propose this material as a new species, *Rossbeevera yunnanensis*, based on morphological evidence as well as phylogenetic analyses of the large subunit of the nuclear ribosomal DNA (nLSU).

Materials & methods

Taxon sampling and macro- and microscopic characterization

Fresh basidiomata were located and collected using a truffle rake. Specimens were placed in wax paper bags and transported to the laboratory within six hours where they were photographed and rapidly dried in a closed container with silica drying beads. The dried specimens are deposited in the Herbarium of Cryptogams, Kunming Institute of Botany, Chinese Academy of Sciences (KUN, with HKAS accession numbers), Kanagawa Prefectural Museum of Natural History (KPM), and the University of Florida Herbarium at the Florida Museum of Natural History (FLAS).

For standard light microscopy and differential interference contrast microscopy, hand-cut sections of dried specimens were mounted in water, 3% KOH, lacto-glycerol, or 1% phloxine B aqueous solution. To determine the amyloid reaction, dried material was stained with Melzer's reagent. Spore dimensions (length $\times$ width range) and their standard deviations (SD), and length of hilar appendages were measured based on 50 randomly selected spores. The length : width ratio (Q) and hilar appendage : spore length ratio (HA/S , newly introduced here) are presented to describe a range of the basidiospore proportion. Measurements include the hilar appendage but not ornamentation or pedicel. Basidium ranges are given as length range $\times$ width.

Molecular methods

DNA was extracted from a dried basidioma using the Indicating FTA Cards (Whatman International Ltd, Maidstone, England). Internal tissue ca. 1 mm thick was soaked in 99.5% ethanol for 2 min before DNA extraction. The extraction was done according to the manufacturer's protocol for plant samples (available at http://www.whatman.com/References/WGI_1397_PlantPoster_V6.pdf) using FTA Cards, more convenient and efficient over traditional methods (Dentinger et al. 2010). Furthermore, we demonstrate here that this technique is useful for dried tissues after a brief ethanol pretreatment. For PCR amplification and sequencing reactions, we used the primer pair LR0R / LR5 (Vilgalys & Hester 1990). For PCR conditions, sequencing and editing of the obtained sequence, see Lebel et al. (2012a). The nLSU sequence is deposited in GenBank.

Sequences included in the nLSU dataset were retrieved from GenBank based on the previous analyses of *Rossbeevera* and allied genera (Lebel et al. 2012a). Some sequences

of *Leccinellum* spp., which are shown to be paraphyletic with *Rossbeevera*, were selected according to the phylogeny in Lebel et al. (2012a) and were used to root the phylogenetic tree. Multiple sequence alignment was performed using Clustal X ver. 1.83 (Thompson et al. 1997) and the data were manually adjusted in SeaView (Galtier et al. 1996). Gaps were treated as “missing” data for all analyses.

The neighbor-joining (NJ) analysis based on p distance was conducted with Clustal X ver. 1.83 with 1000 bootstrap replicates and with default settings. Subsequently, the maximum likelihood (ML) analysis was done by PhyML ver. 3.0 (Guindon et al. 2010) under GTR + I + G model based on hLRT estimated by MrModeltest 2.3 (Nylander 2004), starting the analysis using a tree estimated by the Nearest Neighbor Interchanges (NNIs) and setting the number of bootstrap replicates to 1000. The resulting trees were visualized with FigTree ver. 1.3.1 (Rambaut, available at <http://tree.bio.ed.ac.uk/>).

Results

The finally aligned nLSU dataset was 837 bp long, including full length reads for all 19 sequences but excluding the primer regions. Settings of the best model estimated by hLRT in MrModeltest 2.3 were as follows: base frequencies (A = 0.2700, C = 0.1941, G = 0.2904, T = 0.2455), proportion of invariable sites (I = 0.7687), and gamma distribution shape parameter (α = 1.9606). Maximum likelihood analysis generated one ML tree (ln L = -2078.576887; Fig. 1). Branches that were moderately to strongly supported by ML bootstrap values (>60%) were also strongly supported in the NJ topology (BS >70%). In the *Rossbeevera* clade, *R. eucyanea* and *R. griseovelutina* were each supported to be monophyletic by high BS values but the Australasian species were not well differentiated from each other, as shown in Lebel et al. (2012a). The Chinese *Rossbeevera* sp. MES420 formed a basal branch within the *Rossbeevera* clade and was phylogenetically distinct from the other members of *Rossbeevera* included in this study.

Taxonomy

***Rossbeevera yunnanensis* Orihara & M.E. Sm., sp. nov.**

FIG. 2

MYCOBANK MB 563649

Differs from *Rossbeevera bispora* by thinner or partially lacking peridium, fusoid to fusiform basidiospores, and 2–4-spored basidia.

TYPE: China, Yunnan Province, ChuXiong Yi Autonomous Prefecture, Mt. Zixi, 19 Sept. 2010, M. E. Smith et Z.-W. Ge, MES420 (holotype - HKAS 70689, isotype - KPM-NC0017850, GenBank JN979437).

ETYMOLOGY: *yunnanensis* (Latin) refers to the type locality.

BASIDIOMATA sparse, ca. 1 cm, subglobose, soft, surface covered with very thin, minutely felty, whitish to almost translucent peridium gradually turning bluish gray or black when touched or bruised, the peridium partially absent, exposing the gleba. GLEBA off-white to beige when immature, reddish brown to blackish

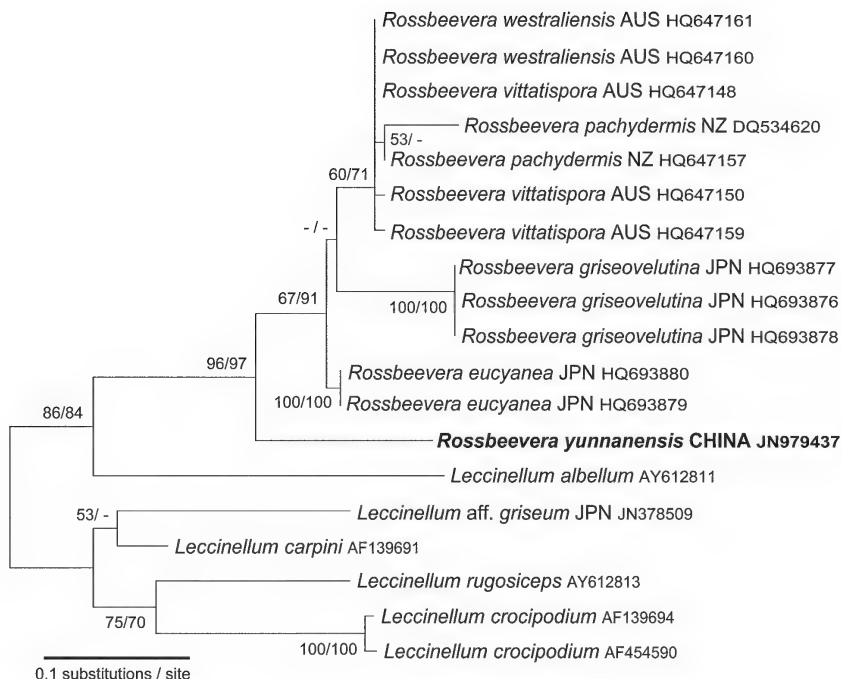


FIGURE 1. Maximum likelihood (ML) tree of the nLSU dataset of *Rossbeevera* species with *Leccinellum* species selected as the outgroup. ML and Neighbor-Joining (NJ) bootstrap (BS) values (1000 replicates; only BS >50% are shown) are indicated above or below branches or at nodes as MLBS/NJBS. Abbreviations: AUS = Australia; NZ = New Zealand; JPN = Japan.

brown at maturity, rubbery, composed of minute, irregular locules, turning bluish gray in some portions when cut and exposed to air, trama subgelatinous, somewhat translucent. STIPE-COLUMELLA present but reduced, somewhat dendroid or as a small basal pad, subgelatinous, translucent. ODOR unknown.

BASIDIOSPORES (14.4–)16.9–23.4(–24) $\times$ 6.8–9(–9.5) μm , (mean $\pm$ SD = 20.1 $\pm$ 1.60 $\times$ 7.8 $\pm$ 0.61), Q = 2–3.8 (mean = 3.1), symmetric, fusoid to fusiform, inamyloid, nondextrinoid, colorless at first then becoming reddish brown at maturity, with 3–4 or rarely 5 smooth, longitudinal ridges up to 2.3 μm high (mostly ca. 1 μm high) in water, walls 0.7–1.5 μm thick, with a large hilar appendage (2–)2.3–3.8 μm long (mean 3.1), HA/S = 0.12–0.22, (mean = 0.16) at the base and a minute, incomplete hollow (> 1 μm in diameter) at the tip. BASIDIA 17.5–30 $\times$ 6.8–11 μm (mean = 23.5 $\times$ 8.4 μm ; n = 15), cylindrical to cylindro-clavate, colorless to pale yellow-brown to reddish brown, 2-, 3- or

4-spored. HYMENIUM developed when immature but collapsed at maturity, colorless, hymenial cystidia not seen; BASIDIOLES cylindrical or clavate to clavulate. SUBHYMENIUM not developed. TRAMA of densely or somewhat loosely interwoven, partly branched, colorless, non-inflated, thin-walled ($< 0.8 \mu\text{m}$ thick), filamentous hyphae $3.5\text{--}8 \mu\text{m}$ broad. STIPE-COLUMELLA composed of loosely interwoven, subgelatinous, thin-walled ($< \text{ca. } 1 \mu\text{m}$ thick) filamentous hyphae $(2\text{--})5\text{--}8\text{--}(9.5) \mu\text{m}$ broad. PERIDIUM thin, absent in some portions, up to $60 \mu\text{m}$ thick in the dried basidioma, yellowish brown under light microscopy, composed of repent, non-inflated, thin-walled ($< 0.8 \mu\text{m}$ thick) filamentous hyphae $2.5\text{--}6 \mu\text{m}$ broad but the hyphae are collapsed in most portions. CLAMP CONNECTIONS absent in all tissues.

HABITAT AND DISTRIBUTION: Hypogeous in mixed forest dominated by *Pinus yunnanensis* Franch., *Lithocarpus mairei* (Schottky) Rehder, *L. dealbatus* (Hook. f. & Thomson ex Miq.) Rehder, and unidentified *Quercus* spp.; known only from Yunnan Province, China.

Discussion

Rossbeevera yunnanensis shares several morphological characteristics with the other *Rossbeevera* species, including the bluish color change of the peridium and reddish brown basidiospores with 3–5 unbranched longitudinal ridges. This new species also has the large, distinctly fusoid to fusiform ($Q = 2.0\text{--}3.8$) basidiospores and remarkably thin peridium characteristic of the genus. *Rossbeevera griseovelutina*, which is reported from Japan, has slightly larger basidiospores ($14.4\text{--}31.9 \times 6.7\text{--}10.4 \mu\text{m}$, mean = $22.2 \times 8.7 \mu\text{m}$) and the Q value is smaller ($Q = 2.0\text{--}3.3$; Lebel et al. 2012a). In addition, the latter species has a more fully developed peridiopellis composed of more or less vertically oriented, partially inflated hyphae. *Rossbeevera yunnanensis* also differs morphologically from another known Chinese species, *R. bispora*, because *R. yunnanensis* has a thinner peridium, larger and narrower basidiospores, and its basidia often bear 4 spores rather than 2 spores characteristic of *R. bispora* (Zhang & Yu 1989). We have examined the holotype of *R. bispora* (GDGM5688) and have confirmed the above-noted differences. The Southeast Asian species, *R. mucosa* is readily distinguished from *R. yunnanensis* in its ellipsoid to fusoid basidiospores ($13\text{--})15\text{--}17 \mu\text{m}$ in diameter with a lower Q value ($Q = 1.76\text{--}2.05$; Lebel et al. 2012a).

The HA/S ratio, which is newly introduced in this study, could be helpful to describe spore shapes objectively when spore sizes vary widely within collections or sporocarps of a particular species. Basidiospores of *R. griseovelutina* are somewhat similar to those of *R. yunnanensis* as discussed above, but the mean HA/S ratio of *R. griseovelutina* was significantly higher than that of *R. yunnanensis*. However, the ranges overlapped: HA/S of *R. griseovelutina* = $0.12\text{--}0.24$, mean 0.18 , $n = 25$ vs. HA/S of *R. yunnanensis* = $0.12\text{--}0.22$, mean

= 0.16. Basidiospore morphology is widely used as a key character for the taxonomy of sequestrate fungi (Castellano et al. 1989). The HA/S difference is proposed as a new, useful diagnostic character that could be useful in the taxonomy of *Rossbeevera* and other sequestrate genera.

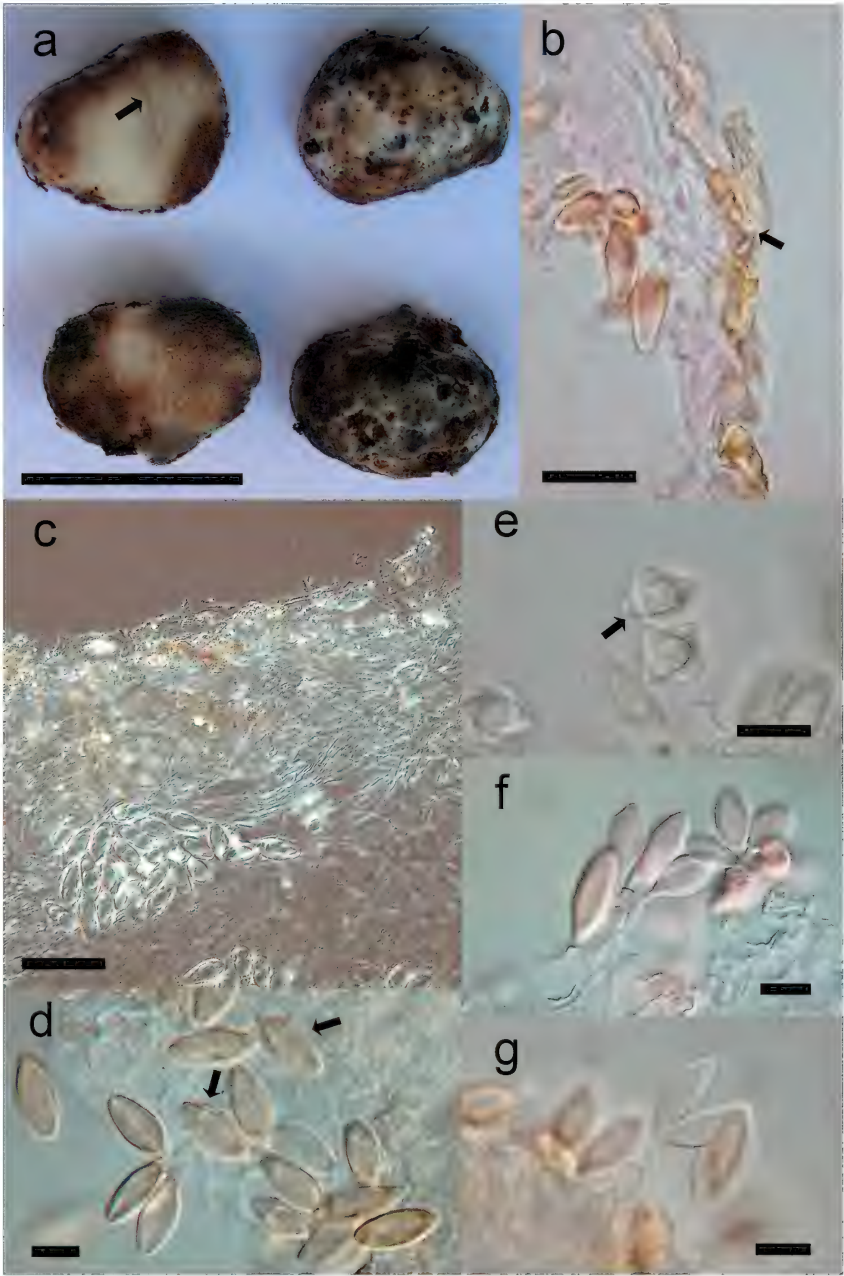
In our phylogeny *R. yunnanensis* formed the earliest diverging lineage within the *Rossbeevera* clade with moderate to strong bootstrap support (ML: 67%; NJ: 91%). Although we have not been able to include sequences of *R. bispora*, it is possible that the two Chinese species are closely related within *Rossbeevera*, because they share a similar overall morphology, including a cutis-like peridium and relatively large basidiospores with mostly 3–4 longitudinal ridges. Sequences from additional genes and specimens of *Rossbeevera* from China and the adjacent regions would help to clarify the evolutionary origin of this sequestrate genus.

Although we have examined only one *R. yunnanensis* collection, its morphological characteristics and unique phylogenetic position are sufficient to discriminate the species from the other members of *Rossbeevera*. We would prefer to have additional specimens in order to provide more ecological information about *R. yunnanensis*, such as details of its phenology and host tree preferences. However, since hypogeous fungi are difficult to find and collect, we felt it was important to describe this new *Rossbeevera* species to promote further research on the systematics of Asian sequestrate fungi. Unique sequestrate fungi have been found in Asia for more than a century (e.g. Corner & Hawker 1953; Smith & Schmull 2011). However, several recent studies highlight the fact that Asia is likely a hotspot for unique, endemic truffles and probably hosts a high diversity of undescribed sequestrate fungi (Yang et al. 2006; Desjardin et al. 2008, 2009; Orihara et al. 2008, 2010, 2012; Kinoshita et al. 2011). Clearly, more taxonomic work on sequestrate fungi is needed in this region.

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FIGURE 2 (right). *Rossbeevera yunnanensis* (holotype): a. Basidiomata. A reduced columella is present in the center of the section (arrow); b. Significantly reduced peridium of dried material (arrow); c. Thick portion of the peridium; d. Mature basidiospores with longitudinal ridges (arrows) mounted in lacto-glycerol; e. Immature basidiospores viewed from above (arrow); f. 3- and 4-spored basidia mounted in lacto-glycerol after a pretreatment with 1% phloxine B aqueous solution; g. 2-spored basidium mounted in lacto-glycerol. Scale bars: a = 1 cm; b = 20 μ m; c = 30 μ m; d–g = 10 μ m.



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***Octaviania violascens*: a new sequestrate bolete from Thailand**

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ABSTRACT — *Octaviania violascens* sp. nov. is proposed for a new sequestrate bolete from Khao Yai National Park, Nakhon Ratchasima Province, Thailand. A comprehensive description and illustrations are provided.

KEY WORDS — Basidiomycota, truffle, Octavianiaceae

Introduction

While walking along an elephant trail collecting mushrooms in a bamboo grove in Khao Yai National Park, Nakhon Ratchasima Province, Thailand, we encountered a rose-colored fungus where recent rain had washed away the topsoil. The distinctive characteristics of this species, besides the rose color of its basidiocarps, were that it bruised deep reddish violet, contained non-glutinous gleba, and had clamp connections in the peridial layer and basidia containing only two highly ornamented spores. Its micromorphology clearly placed it in *Octaviania* and it is the first record of this genus from Thailand. *Octaviania* is included in the group of sequestrate basidiomycetes called truffles. In Thailand 8 species of sequestrate fungi have been reported, including *Arcangeliella rosea* (Harkn.) Zeller & C.W. Dodge (Ellingsen 1982), *A. beccarii* (Petri) Zeller & C.W. Dodge (Chandrasrikul et al. 2008), *Descomyces* cf. *albellus* (Masse & Rodway) Bougher & Castellano (Dissing 1963, Ellingsen 1982), *Mycoamaranthus cambodgensis* (Pat.) Trappe, et al. (Chandrasrikul et al. 2007, 2008, Lumyong et al. 2003), *Radiigera tropica* Orihara & T. Kasuya, and three other unknown species from Dissing (1963) in the genera *Hymenogaster*,

Leucogaster, and *Melanogaster*. This paper describes a newly discovered species, *Octaviania violascens*, from Thailand.

Materials & methods

COLLECTION SITE: The study site is in a bamboo grove, in a secondary forest near the 28 km marker post along the road from the northern entrance in Khao Yai National Park, Nakhon Ratchasima Province, Thailand.

FUNGAL MATERIALS: Dried specimens are deposited in the BIOTEC Bangkok Herbarium (BBH).

MORPHOLOGY: Macromorphological data were recorded from dried specimens using an Olympus model XZ30 stereomicroscope. Micromorphological data were recorded from dried specimens using an Olympus model BX51 microscope. Drawings were made using an Olympus Camera Lucida model U-DA. Measurements and drawings were made from slide preparations stained with cotton blue, Melzer's reagent, or 3% potassium hydroxide. The following abbreviations are used for chemical reactions: IKI (Melzer's reagent, with IKI- = non-amyloid and non-dextrinoid; IKI+ = amyloid or dextrinoid), KOH (3% potassium hydroxide), and CB (cotton blue; CB+ = cyanophilous; CB(+) = weakly cyanophilous; CB- = acyanophilous). Basidium width was measured at the broadest part, and basidium length was measured from the apex (sterigmata excluded) to basal septum. Color terms and notations in parentheses follow Kornerup and Wanscher (1963). Spore statistics include: $\bar{x}$ = the arithmetic mean of the spore length by spore width ($\pm$ standard deviation) for n spores measured; Q = the quotient of length and width in any one spore, indicated as a range of variation in n spores measured; Q_m = the mean of Q -values ($\pm$ standard deviation). Ultrastructures were recorded from dried specimens using a Hitachi S-3400N scanning electron microscope.

Taxonomy

Octaviania violascens Choeyklin, Boonprat. & Somrith., sp. nov. PLATES 1–3

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Differt a O. purpurea, absque columella et rhizomorpha, loculus rotundatus, spora verruca angulari, basidia bispora pariete crasso; praesentia fibularum et cellularum spheropeduncularum et hypharum laticiferarum.

TYPE – THAILAND. Nakhon Ratchasima, Pak Chong district, Khao Yai National Park, 28 km. marker post (*Bambusa* spp. forest), 14°30'59.47"N 101°22'08.29"E, 23 August 2010, coll. Rattaket Choeyklin & Sujinda Sommai, RCK 00093 (**Holotype** BBH 30342).

ETYMOLOGY – *violascens* (Latin) = turning violet.

Basidiocarps 9–18 mm tall $\times$ 12–22 mm wide (dried specimens), ovoid, subglobose to turbinate, white to cream colored near base, lumpy or folded on the upper part; smooth to cracking to form squamules, the squamules reddish grey to greyish rose (12B2–3), staining reddish violet to deep violet (18C–D8) where bruised or cut; dull, dry, hard and cartilaginous. Peridium thin, 0.5–0.6 mm diam., white, quickly changing from violet white to pale violet (16A3–4) when cut. Gleba lacunate, 0.5–1 mm diam., locules filled with powdery brown



PLATE 1: *Octaviania violascens*. 1. Basidiocarps in various shapes; 2. Peridial layer cracking to form squamules and deep violet stains where bruised; 3. Gleba lacunate, quickly changing to violet brown when cut, basidiocarps without rhizoid (holotype). Bar = 10 mm.

spores when dried, dark brown when fresh, solid when fresh and with a hollow central cavity when dried, watery, quickly changing to violet brown (11F8) when cut; sterile tissue between locules fibrous, white but immediately changing to reddish violet to deep violet when bruised (18C–D8). Latex absent. Columella absent. Odor not distinctive.

Basidiospores without spines $(11-12-15(-19) \times 11-14(-15) \mu\text{m}$ ($x = 13.84 \pm 1.53 \times 12.44 \pm 1.16 \mu\text{m}$, $Q = 1.0-1.46$, $Q_m = 1.09 \pm 0.11$, $n = 25$); with spines $(12-15-17(-18) \times (10-15-20) \mu\text{m}$ ($x = 15.79 \pm 1.36 \times 16.40 \pm 2.14 \mu\text{m}$, $Q = 0.67-1.50$, $Q_m = 0.98 \pm 0.15$), orthotropic and heterotropic, symmetric and asymmetric, globose to subglobose, walls $2.0-2.5 \mu\text{m}$ thick, pale yellowish brown, golden-brown, brown to dark brown in water and 3% KOH, IKI+ (weakly dextrinoid), CB-, spore ornamentation echinulate to conical spines, $1-2 \mu\text{m}$ tall $\times 2 \mu\text{m}$ wide at base $\times 1 \mu\text{m}$ wide at apex, most apical spines acute

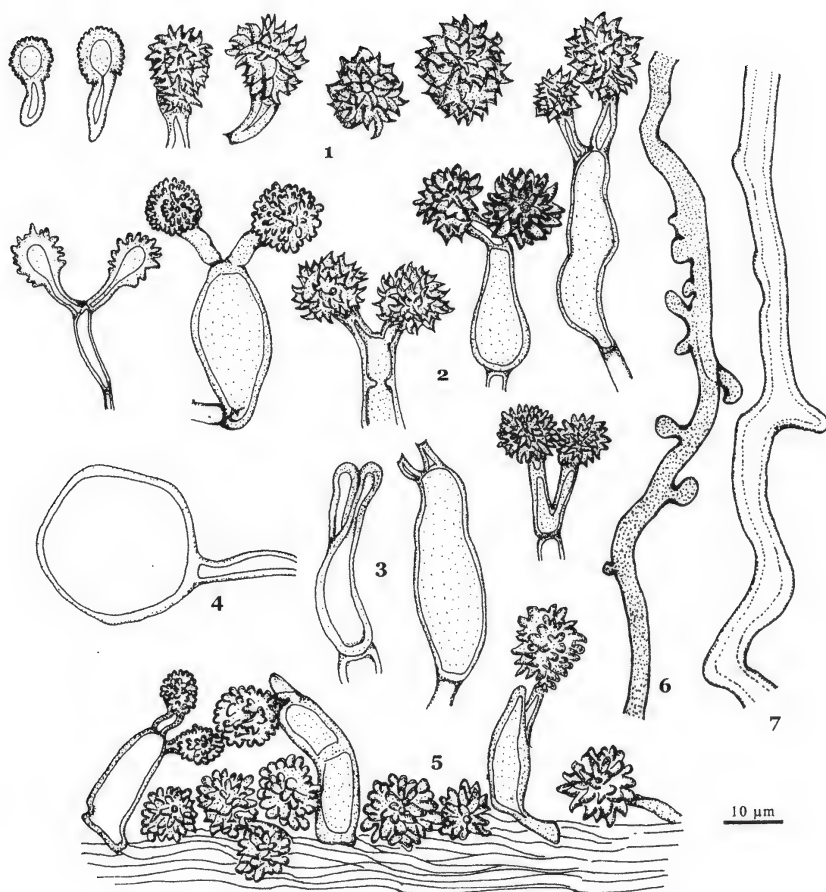


PLATE 2: *Octaviania violascens* (holotype). 1. Basidiospore formation; 2. 2-spored basidia; 3. Basidia shapes; 4. Spheropedunculate cell from peridial context; 5. Hymenium with basidia; 6. Oleiferous hyphae; 7. Skeletal hyphae. Bar = 10 μ m.

but some blunted or slightly bent, hyaline in 3% KOH and water, IKI+ (weakly dextrinoid), CB+; pedicel 6–10 $\times$ 3 μ m, walls 1–1.5 μ m thick, pale yellowish-brown. Basidia (12.5–)20–31(–35) $\times$ (5–)7.5–15 μ m, 2-spored, lageniform to fusoid, walls >1 μ m thick, hyaline when young to pale yellowish brown to brown when mature in water and KOH, IKI–, CB+, basally septate; sterigmata 7–8 μ m tall $\times$ 3 μ m wide, walls 1 μ m thick, pale brown in water and 3% KOH, IKI–, CB+, septa present at the sterigmal base. Peridial context 170–300 μ m diam., containing two types of hyphae consisting of oleiferous and binding hyphae; oleiferous hyphae 3–4 μ m wide, thin-walled, brown in water and 3%

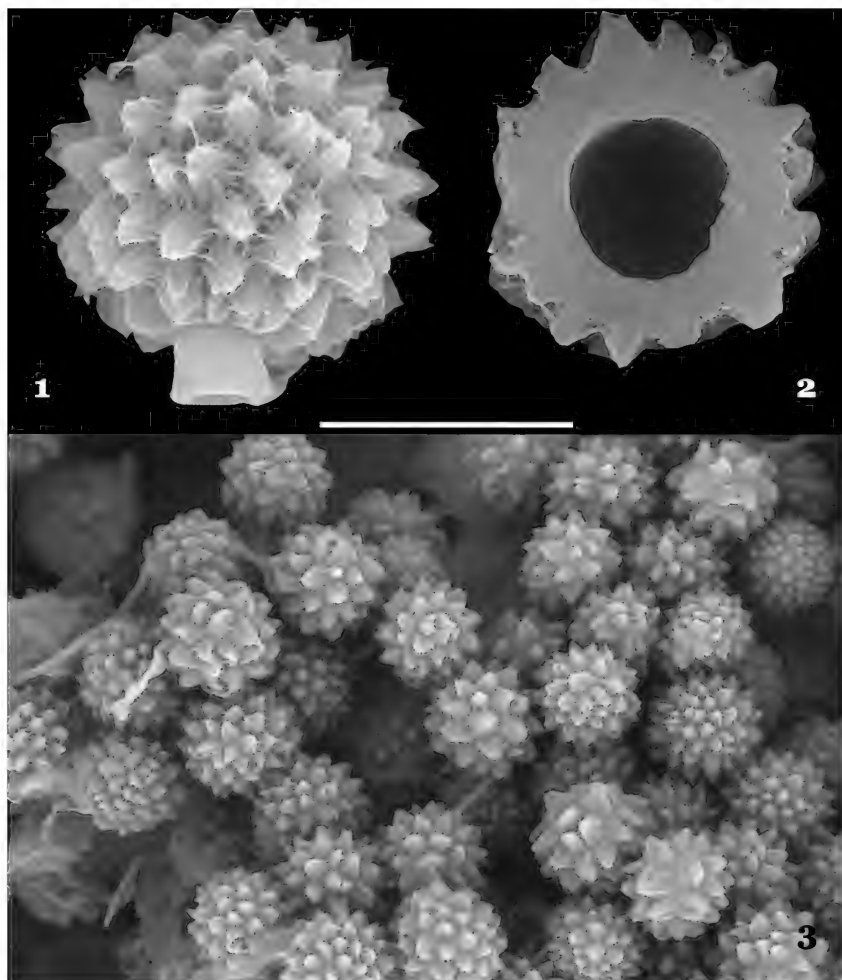


PLATE 3: *Octaviania violascens* (holotype): scanning electron micrographs of spores. 1. Mature basidiospore, 2. Basidiospore wall, 3. Basidiospores variation, Bar 1–2 = 10 μm , 3 = 3 μm

KOH, IKI–, CB+; binding hyphae 4–6 μm width, thick-walled, walls 1–1.5 μm thick, hyaline in water and 3% KOH, IKI–, CB+, and pinkish when stained with phloxine. Sphaeropedunculate cells 27.5–30 $\times$ 27.5–30 μm , embedded in the peridial context, pedicel 7 $\times$ 2 μm , ovoid, thick-walled, hyaline, IKI–. Subhymenium IKI+ (weakly dextrinoid). Tramal plate 25–30 μm diam., thin-walled, hyaline, IKI–. Peridiopellis of repent hyphae, interwoven, thin-walled, hyaline, IKI–. Sterile tissues that separate the locules are identical to the tramal plate. Clamp connections present only in the peridial tissue.

ECOLOGY & DISTRIBUTION: Solitary to gregarious or in small clusters, subhypogeous to hypogeous, in sandy soil in a *Bambusa* spp. (*Bambusaceae*) forest, in open secondary forest. Thailand, Nakhon Ratchasima Province, Khao Yai National Park, 28 km marker post.

Discussion

Octaviania violascens may be diagnosed by its echinulate spores with conical spines and thick-walled 2-spored basidia. The new species is most similar to *O. purpurea* Coker & Couch (Coker & Couch 1928) but differs in the texture of the gleba, absence of a columella and rhizomorphs, spore shape, colour, and ornamentation, presence of laticiferous hyphae, and basidia shape and sterigmata number (TABLE 1). Spheropedunculate cells in the peridial context and clamp connections found in *O. violascens* are absent in *O. purpurea* (TABLE 1).

Heliogaster Orihara & K. Iwase also produces an empty-chambered nonglutinous gleba, but unlike *Octaviania* its gleba is soft, not rubbery and its peridium composed of filamentous hyphae (not partly inflated hyphae with isodiametric cells) and spores with a perisporium. In the absence of DNA analyses, we retain *O. violascens* within *Octaviania* based on morphology.

TABLE 1. Morphological characters in *Octaviania violascens* and *O. purpurea*.

MORPHOLOGY	<i>O. violascens</i>	<i>O. purpurea</i>
Sporocarp texture	Rubbery	Not reported
Spore ornamentation	Echinulate with conical spines	Angular warts
Spore shape	Globose	Subglobose
Spore color	Pale yellowish brown to brown	Yellowish brown to dark brown
Basidia	2-spored, thick-walled	1-spored, thin-walled
Basidial shape	Lageniform to fusoid	Jug-shaped
Basidial size	~20–31 × 7.5–15 µm	30–44 × 5.5–7.8 µm
Clamp connection	Present	Absent
Spheropedunculate cells	Present	Absent
Rhizoid & columella	Absent	Present
Locules	Round	Labyrinthiform

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We express appreciation to Michael Castellano and Teresa Lebel for reviewing this paper. We thank Tim Flegel for reviewing English. We are grateful Ellen Bloch (curator of New York Botanical Garden) for the loan of *Octaviania purpurea* Coker & Couch #780569. This study was funded by BIOTEC, Thailand (TRF/BRT – The biodiversity Research and Training Program grant #BRT_R650001) for field survey. We appreciate the Department of National Parks, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment for permitting collecting inside Khao Yai National Park.

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New records of cercosporoid hyphomycetes from IranMAHDI PIRNIA^{1*}, RASOUL ZARE²,HAMID R. ZAMANIZADEH¹ & AKBAR KHODAPARAST³¹Science and Research Branch, Islamic Azad University, Tehran, Iran²Department of Botany, Iranian Research Institute of Plant Protection,
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ABSTRACT — Eight collections from different localities in the northern provinces of Iran were obtained during spring-summer 2010–11. Seven species on eight host plants were identified: *Cercospora pantoleuca* on *Plantago lanceolata*, *Cercospora primulae* on *Primula macrocalyx*, *C. virgaureae* on *Conyza bonariensis*, *Passalora bondartsevii* on *Medicago* sp., *Pseudocercospora danaicola* on *Danae racemosa*, *P. paraguayensis* on *Eucalyptus camaldulensis*, and *Scolecostigmina confluens* on *Crataegus melanocarpa* and *C. pseudomelanocarpa*. These taxa are new records to Iran. *Cercosporina danaicola* is recombined as *Pseudocercospora danaicola*.

KEY WORDS — *Cercospora*-like fungi, taxonomy, Asia Minor, leaf spot pathogens

Introduction

Cercosporoid fungi include over 30 morphologically similar anamorphic hyphomycetous genera that are mainly associated with leaf spots on various host plants such as cereals, vegetables, ornamentals, forest trees, and weeds (Crous & Braun 2003). *Cercospora* Fresen. is one of the largest genera in this group. In his monograph of *Cercospora*, Chupp (1954) listed over 1800 species names and accepted 1419 species. He adopted a broad generic concept and mainly considered the characteristics of conidia (hilum thickness, pigmentation, number, and arrangement). In a major taxonomic treatment, Deighton (1967, 1973, 1974, 1976, 1979) divided and reclassified many *Cercospora* species into several segregate genera, including *Cercospora* Sacc., *Cercosporidium* Earle, *Paracercospora* Deighton, *Pseudocercospora* Speg., *Pseudocercospora* Deighton, and *Pseudocercosporidium* Deighton. Other morphologically similar fungi are members of *Passalora* Fr. and *Scolecostigmina* U. Braun. *Cercospora* species are characterized by acicular hyaline septate conidia with conspicuous hila produced

on pigmented unbranched septate smooth conidiophores. *Cercosporella* species are easily distinguished from *Cercospora* species by colourless structures with thickened darkened conidiogenous loci. In *Pseudocercospora*, conidiogenous loci and hila are inconspicuous, not thickened, and not darkened. *Passalora* is distinguished by pigmented conidiophores and pigmented ellipsoid-fusiform to obclavate-subcylindrical conidia. *Scolecotigmina* is diagnosed by thick-walled verruculose conidiophores with conspicuous annellations in the upper part. Conidia in this genus are subcylindric-obclavate, smooth to verruculose, transversely multi-euseptate, and occasionally with a few longitudinal septa. Crous & Braun (2003) published an annotated checklist for *Cercospora* and *Passalora* names with 5720 taxa and taxonomic re-allocations of numerous species. Recently Braun & Crous (2007) proposed several new species, combinations, and names after re-examining the type collections of *Cercospora* species and other related genera.

Iranian records of *Cercospora* and other related genera have been only poorly studied. Scharif & Ershad (1966) presented a list of fungi on various host plants including a few *Cercospora* and *Pseudocercospora* species. Ershad (1990, 2000, 2002) reported four *Cercospora* species from Iran and later (Ershad 2009) corrected 59 *Cercospora* names previously published from Iran, partly according to Crous & Braun (2003), thus reducing the number of species to 22 with 14 uncertain species. He did not follow Crous & Braun (2003) in merging morphologically indistinguishable taxa on various host plants in *Cercospora apii* s. lat. Recently Pirnia et al. (2010, 2012a, 2012b) studied *Cercospora*, *Passalora*, *Ramularia*, and *Ramularia*-like species in Iran following the species concepts proposed by Crous & Braun (2003) and Braun (1998). They linked six host plants with *C. apii* s. lat. Furthermore, some specimens deposited in the fungus reference of the Iranian Ministry of Agriculture (Iranian Research Institute of Plant Protection, Tehran) have been examined and proved to be morphologically indistinguishable from *C. apii*.

Materials & methods

Specimens with leaf spot symptoms from different localities in northern Iran (i.e., Guilan, Mazandaran, Golestan, northern Khorasan provinces) were collected during spring-summer 2010–11. Microscopic slides were prepared from stromata, conidiophores, and conidia in 25% lactic acid. Species were identified based on stromata (presence/absence) and their development, conidia (pigmentation, shape, dimensions), hila (thickness, darkness), conidiophores (pigmentation, dimensions) and conidial scars (position, thickness, darkness). Drawings were made using a drawing tube attached to an Olympus BH-2 microscope.

Results

In our study on cercosporoid hyphomycetes of Iran, we identified 70 taxa on 100 host plants (TABLE 1). Of the 21 taxa that are new records for the mycobiota

TABLE 1. Cercosporoid hyphomycetes and hosts identified from Iran

PATHOGEN	Hosts
* <i>Cercospora acnidae</i>	<i>Amaranthus chlorostachys</i> var. <i>chlorostachys</i>
<i>C. althaeina</i>	* <i>Gossypium hirsutum</i>
* <i>C. apii</i>	<i>Abutilon theophrasti</i> , <i>Euphorbia heterophylla</i> , <i>Gerbera jamesonii</i> , <i>Medicago</i> sp., <i>Pelargonium zonale</i> , <i>Petunia hybrida</i> , <i>Solanum lycopersicum</i> , <i>Vigna sinensis</i> , <i>Zantedeschia aethiopica</i>
<i>C. beticola</i>	<i>Beta vulgaris</i> , <i>B. maritima</i>
* <i>C. bizzozzeriana</i>	<i>Cardaria draba</i>
* <i>C. caricis</i>	<i>Carex orbicularis</i>
<i>C. cheiranthi</i>	<i>Cheiranthus cheiri</i>
<i>C. lactucae-sativae</i>	<i>Lactuca sativa</i> , * <i>L. serriola</i>
* <i>C. mercurialis</i>	<i>Mercurialis annua</i>
* <i>C. pantoleuca</i>	<i>Plantago lanceolata</i>
* <i>C. peckiana</i>	<i>Rumex sanguineus</i> , <i>R. crispus</i>
<i>C. sorghi</i>	* <i>Sorghum halepense</i>
<i>C. traversiana</i>	<i>Trigonella foenum-graecum</i>
<i>C. violae</i>	<i>Viola sylvestris</i> , <i>Viola</i> sp.
<i>C. zebrina</i>	<i>Medicago</i> sp.
<i>C. zonata</i>	<i>Vicia faba</i>
* <i>Cercospora primulae</i>	<i>Primula macrocalyx</i>
* <i>C. virgaureae</i>	<i>Conyza bonariensis</i>
<i>Neovularia ovata</i>	<i>Salvia hypoleuca</i> , <i>S. limbata</i> , <i>S. nemorosa</i>
<i>Passalora bolleana</i>	<i>Ficus carica</i>
* <i>P. bondartsevii</i>	* <i>Medicago</i> sp.
<i>P. calotropidis</i>	<i>Calotropis procera</i>
* <i>P. chaetomium</i>	<i>Euphorbia marschalliana</i>
<i>P. circumscissa</i>	<i>Cerasus vulgaris</i> , <i>Prunus domestica</i>
<i>P. cousiniae</i>	<i>Cousinia</i> sp.
<i>P. dubia</i>	* <i>Chenopodium album</i>
<i>P. graminis</i>	* <i>Agropyron</i> sp., * <i>Poa annua</i> , * <i>Stipa</i> sp.
<i>P. microsora</i>	<i>Tilia begoniifolia</i>
<i>P. personata</i>	<i>Arachis hypogaea</i>
<i>P. phaeopappi</i>	<i>Phaeopappus aucheri</i>
<i>P. punctum</i>	<i>Anethum graveolens</i> , <i>Foeniculum vulgare</i> , <i>Petroselinum sativum</i>
<i>P. rosae</i>	* <i>Rosa persica</i> , <i>Rosa</i> sp.
<i>P. rosicola</i>	<i>Rosa</i> sp.
* <i>P. ziziphi</i>	<i>Ziziphus spina-christi</i>
<i>Pseudocercospora abelmoschi</i>	<i>Hibiscus cannabinus</i>
<i>P. atromarginalis</i>	<i>Solanum nigrum</i>
<i>P. cruenta</i>	<i>Vigna sinensis</i>
* <i>P. danaicola</i>	<i>Danae racemosa</i>
* <i>P. griseola</i>	<i>Phaseolus vulgaris</i>
* <i>P. heteromalla</i>	<i>Rubus</i> sp.
<i>P. jujubae</i>	<i>Ziziphus spina-christi</i>
<i>P. kaki</i>	<i>Diospyros lotus</i> , <i>D. kaki</i>
<i>P. neriella</i>	<i>Nerium oleander</i>
* <i>P. paraguayensis</i>	<i>Eucalyptus camaldulensis</i>
<i>P. punicae</i>	<i>Punica granatum</i>

Table 1, concluded

PATHOGEN	HOSTS
<i>P. rubi</i>	<i>Rubus</i> sp.
* <i>P. salicina</i>	<i>Salix alba</i>
<i>P. salvadorae</i>	<i>Salvadora persica</i>
<i>P. sphaerellae-eugeniae</i>	<i>Eugenia jambos</i>
<i>P. vitis</i>	<i>Vitis sylvestris</i> , <i>V. vinifera</i>
<i>Ramularia anchusae</i>	<i>Anchusa italica</i> , <i>A. ovata</i>
<i>R. brunnea</i>	<i>Tussilago farfara</i>
<i>R. cynarae</i>	<i>Carthamus oxyacantha</i> , <i>C. tinctorius</i>
<i>R. geranii</i> var. <i>geranii</i>	<i>Geranium pyrenaicum</i>
<i>R. grevilleana</i> var. <i>grevilleana</i>	<i>Fragaria x ananassa</i> , <i>Potentilla reptans</i>
<i>R. heraclei</i>	<i>Heracleum persicum</i>
* <i>R. inaequalis</i>	* <i>Calendula persica</i>
<i>R. lamii</i> var. <i>lamii</i>	* <i>Mentha piperita</i> , <i>Mentha</i> sp.
* <i>R. macularis</i>	<i>Chenopodium album</i>
* <i>R. pratensis</i> var. <i>pratensis</i>	<i>Rumex crispus</i> , <i>Rumex</i> sp.
<i>R. rhabdospora</i>	<i>Plantago lanceolata</i>
<i>R. rubella</i>	* <i>Rumex conglomeratus</i>
<i>R. rumicis</i>	<i>Rumex crispus</i>
<i>R. sambucina</i>	<i>Sambucus ebulus</i>
<i>R. simplex</i>	<i>Ranunculus oxyspermus</i> , <i>R. sahendicus</i>
<i>R. uredinicola</i>	<i>Uredinia</i> of <i>Melampsora</i> sp. on <i>Salix babylonica</i>
<i>R. urticae</i>	<i>Urtica dioica</i> , <i>Urtica urens</i>
<i>Ramulariopsis gossypii</i>	<i>Gossypium hirsutum</i>
* <i>Scolecotigmina confluens</i>	<i>Crataegus melanocarpa</i> , <i>C. pseudomelanocarpa</i>
<i>Sirosporium celtidis</i>	<i>Celtis australis</i>

* = new pathogen and host records

of Iran, seven are treated in detail in this paper. All specimens are deposited in the fungus reference of Iranian Ministry of Agriculture "IRAN" at the Iranian Research Institute of Plant Protection. Synonyms are listed in Crous & Braun (2003) and Braun (1995, 1998).

Taxonomy

Cercospora pantoleuca Sacc., Michelia 1: 268 (1878)

FIG. 1

Leaf spots circular, numerous, brown to blackish brown with gray center, 1–3 mm in diameter; caespituli amphigenous, mostly epiphyllous, punctiform; stromata small to fairly prominent, brown, 20–25 µm wide; conidiophores fasciculate, 8–15 stalks, arising through stomata, pale olivaceous-brown, paler towards the tip, aseptate or with few inconspicuous septa, straight, subcylindrical to geniculate-sinuuous, not branched, smooth, thin, 15–50 × 2.5–5 µm; conidial scars conspicuous, thickened and darkened, terminal and lateral, 1.5–2 µm wide; conidia formed singly, hyaline, acicular, narrowly obclavate, straight to

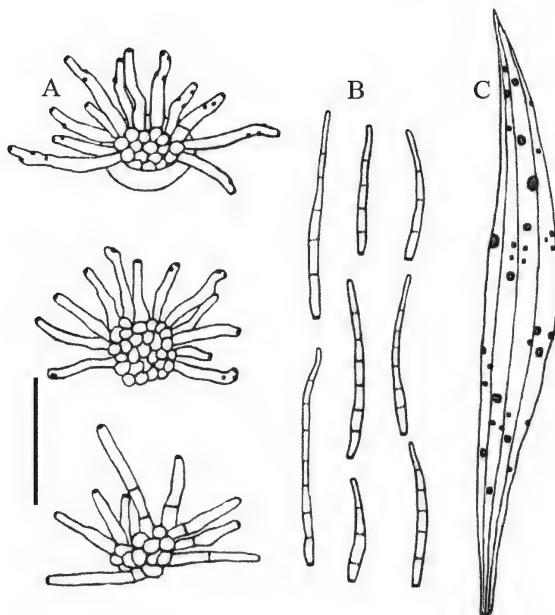


FIG. 1. *Cercospora pantoleuca* on *Plantago lanceolata*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 μm).

slightly curved, smooth, thin, 3–7-septate, base truncate, tip obtuse to subacute, $40\text{--}90 \times 2.5\text{--}4$ (–5) μm ; hilum thickened and darkened.

SPECIMEN EXAMINED: IRAN, NORTHERN KHORASAN PROVINCE, Shirvan, on *Plantago lanceolata* L., 3 July 2011, B. Bicharanlou (IRAN 15498 F).

NOTE—This species is characterized by short conidiophores and conidia. Conidiophores are pale olivaceous-brown to subhyaline, and conidial scars are conspicuous, terminal, and lateral. These characters distinguish this species from other *Cercospora* species. The Iranian specimen examined closely resembles Braun's (1995) description of this species but slightly differs in having faintly pigmented conidiophores.

Cercosporella primulae Allesch., Ber. Bayer. Bot. Ges. 2: 18 (1892)

FIG. 2

Leaf spots circular to subcircular, yellowish, ochraceous to brown, margin indefinite, surrounded by a yellowish halo, 2–8 mm in diameter; caespituli amphigenous, punctiform, whitish; stromata substomatal to intraepidermal, composed of few swollen hyphal cells, colourless; conidiophores in small fascicles, 3–5 stalks, rarely solitary, arising through stomata or erumpent through the cuticle, hyaline, straight to geniculate-sinuous, not branched, smooth, thin-

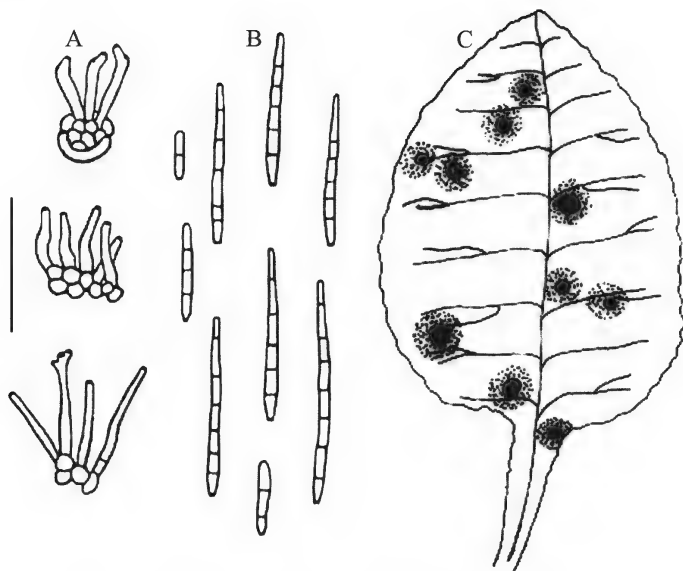


FIG. 2. *Cercospora primulae* on *Primula macrocalyx*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 μ m).

walled, 0–1-septate, 20–45 $\times$ 2.5–5 μ m; conidial scars conspicuous, thickened; conidia formed singly, hyaline, obclavate-acicular to subcylindrical, smooth, thin, indistinctly multiseptate, 0–7-septate, base truncate to obconically truncate, tip subacute, 20–85 $\times$ 3–5 μ m; hilum slightly thickened and darkened.

SPECIMEN EXAMINED: IRAN, GOLESTAN PROVINCE, Gorgan, on *Primula macrocalyx* Bunge, 13 May 2011, M. Pirnia (IRAN 15479 F).

NOTE—This taxon is characterized by moderately short and hyaline conidiophores and conidia. Because of its hyaline conidiophores and conidia as well as the structure of the scars, Braun (1995) maintained this species in *Cercospora*.

Cercospora virgaureae (Thüm.) Allesch., Hedwigia 34: 286 (1895)

FIG. 3

Leaf spots subcircular to irregular, at first yellowish brown, later grayish brown, 2–6 mm in diameter; caespituli hypophyllous, whitish to grayish white, punctiform to dense; stromata substomatal, composed of some aggregated swollen hyphal cells, conidiophores fasciculate, 3–8 stalks, arising from stromata, emerging through stomata, hyaline, below straight, geniculate-sinuous towards the apex, not branched, 0–1-septate, 25–65 $\times$ 4–8 μ m; conidiogenous cells integrated, terminal; conidial scars conspicuous, thickened and darkened, terminal and lateral; conidia formed singly, hyaline, subcylindrical to obclavate,

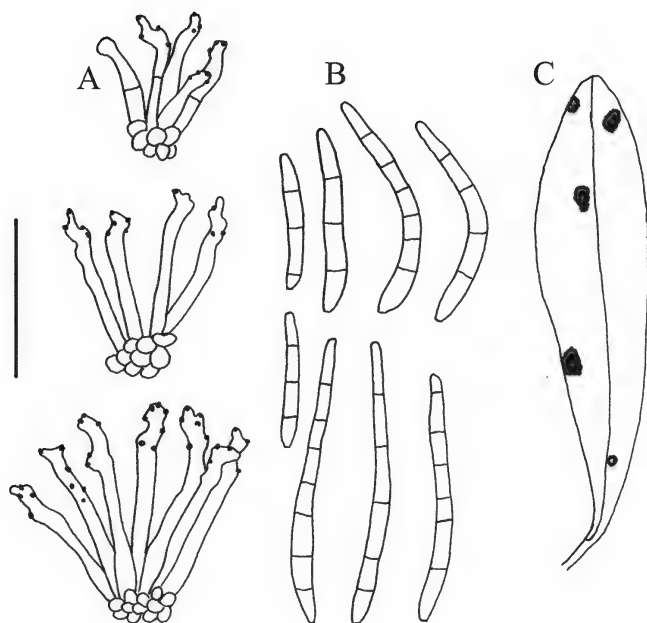


FIG. 3. *Cercospora virgaureae* on *Conyza bonariensis*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 μ m).

straight to slightly curved, smooth, thin, multiseptate, 3–7 septa, base truncate to obconically truncate, tip mostly obtuse, $40\text{--}95 \times 4\text{--}7 \mu\text{m}$; hilum distinct.

SPECIMEN EXAMINED: IRAN, GUILAN PROVINCE, Lahijan, on *Conyza bonariensis* (L.) Cronquist, 29 Nov. 2010, M.R. Mirzaee (IRAN 15480 F).

NOTE—Morphology of the specimen examined agrees with the description provided by Braun (1995). *Cercospora virgaureae* is widespread and has a wide host range on many genera of Asteraceae. This taxon is well characterized by hyaline to subhyaline structures, long conidiophores and conidia, and numerous conspicuous conidial scars on conidiophores.

Passalora bondartsevii U. Braun & Melnik, Trudy Bot. Inst. im. V.L. Komarova 20: 43 (1997)

FIG. 4

Leaf spots circular to irregular, pale brown, surrounded by a yellowish halo, 5–10 mm in diameter; caespituli epiphyllous, punctiform; conidiophores in small to dense fascicles, 7–20 stalks, arising from stromata, brown, erect, subcylindrical, slightly geniculate-sinuous towards the apex, smooth, thin, continuous to septate, sometimes constricted at the septa, $40\text{--}90 \times 4\text{--}5 \mu\text{m}$;

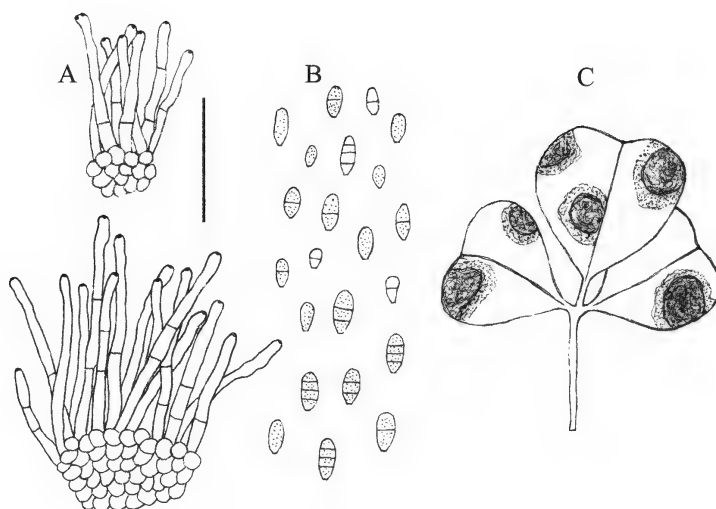


FIG. 4. *Passalora bondartsevii* on *Medicago* sp.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 μ m).

conidiogenous cells integrated, terminal; conidial scars moderately thickened and darkened; conidia formed singly, olivaceous-brown, ellipsoid, obovoid, smooth to rough, 0–3-septate, base obconically truncate, apex obtuse to rounded, $10\text{--}20 \times 5\text{--}7.5 \mu\text{m}$; hilum darkened, unthickened to slightly thickened.

SPECIMEN EXAMINED: IRAN, MAZANDARAN PROVINCE, Babolsar, on *Medicago* sp., 25 June 2011, M. Rafiee (IRAN 15499 F).

NOTE—This taxon was originally established by Braun & Melnik (1997) on *Onobrychis vicifolia* Scop., from Russia and is only known from the type collection. *Passalora bondartsevii* is diagnosed by ellipsoid-obovoid to obclavate-subcylindrical, smooth to faintly rough conidia. In our specimen the conidia are ellipsoid, obovoid, and mostly rough, but we did not see any obclavate-subcylindrical conidia. However, other characters agree well with the description in Braun & Melnik (1997). Therefore, we assign the Iranian collection tentatively to *P. bondartsevii* even though another host genus is involved.

***Pseudocercospora danaicola* (Vienn.-Bourg.) Pirnia & Zare, comb. nov.**

FIG. 5

MYCOBANK MB 563391

= *Cercosporina danaicola* Vienn.-Bourg. Ann. Phytopathol.
2(4): 689 734 (1971 ["1970"]) as "*danaecola*".

Leaf spots circular to subcircular, sometimes with spacious blight specially at the tip of leaves, reddish brown, 10–15 mm in diameter; caespituli amphigenous,

punctiform; stromata present, globular, well developed, dark brown, 45–60 μm wide; conidiophores in dense fascicles, arising from stromata, pale olivaceous-brown, subcylindrical, sinuous, rarely once geniculate, not branched, smooth, thin, aseptate, 8–30 $\times$ 2–3 μm ; conidiogenous loci inconspicuous; conidial scars unthickened and inconspicuous; conidia formed singly, pale olivaceous, acicular, straight to slightly curved, smooth, thin, indistinctly 1–5-septate, base truncate, tip subacute, 65–130 $\times$ 2.5–4 μm ; hilum inconspicuous, unthickened and not darkened.

SPECIMENS EXAMINED (deposited as "*Cercosporina danaicola*"): **IRAN, GOLESTAN PROVINCE**, Khan-behin, Shirabad forest, on *Danae racemosa* Moench, 7 May 1976, D. Ershad (IRAN 515 F); **MAZANDARAN PROVINCE**, Nowshahr, on *D. racemosa*, 19 Aug. 1973, collector unknown (IRAN 516 F, **neotype**, designated here).

NOTE—The name *Cercosporina danaicola* was originally published by Viennot-Bourgin based on material from Iran (Viennot-Bourgin et al. 1971). Careful microscopic examination of morphological characteristics showed that the conidiogenous loci and hila are inconspicuous, unthickened, and not darkened. Crous & Braun (2003) place species with these morphological features into *Pseudocercospora*. Type material could not be traced, but based on the Iranian

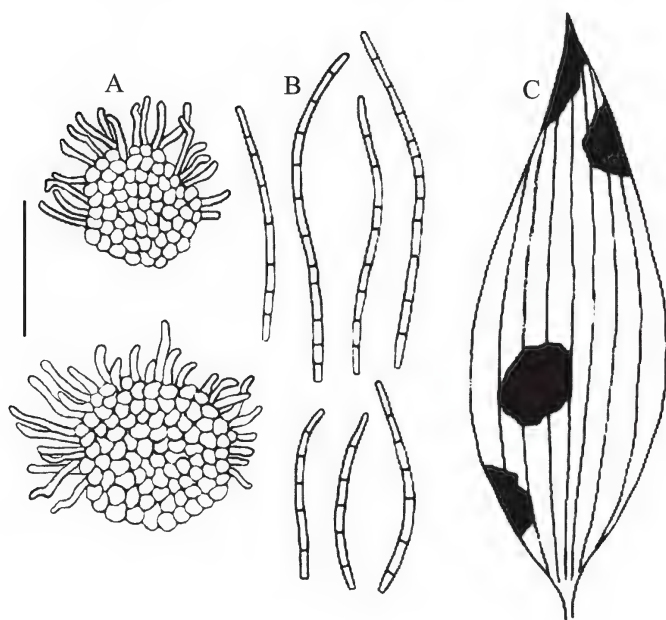


FIG. 5. *Pseudocercospora danaicola* on *Danae racemosa*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 μm).

specimens examined, this species can be transferred to *Pseudocercospora*. The collection IRAN 516 is designated as neotype.

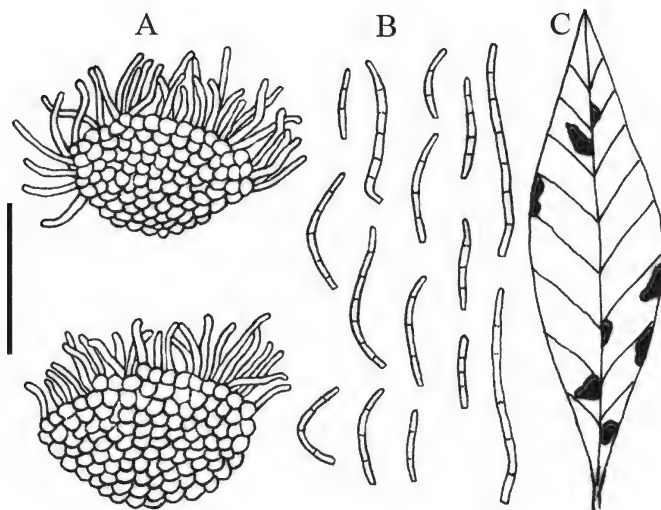


FIG. 6. *Pseudocercospora paraguayensis* on *Eucalyptus camaldulensis*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 µm).

Pseudocercospora paraguayensis (Tak. Kobay.) Crous, Mycotaxon 57: 270 (1996)

FIG. 6

Leaf spots irregular, vein-limited, pale brown to dark grayish brown, border slightly raised, 2–5 mm in diameter, sometimes coalescing into a large blotch; caespituli amphigenous, mostly hypophyllous, punctiform; stromata present, well developed, brown, 50–70 µm wide; conidiophores densely fasciculate, compact, subhyaline to pale olivaceous, subcylindrical, aseptate, not branched, sinuous to rarely once geniculate, straight to curved, rounded at the apex, 10–30 × 2–3 µm; conidial scars unthickened and inconspicuous; conidia formed singly, subhyaline or very pale olivaceous, narrowly obclavate, straight to curved, indistinctly 2–6-septate, apex subacute or subobtuse, base truncate to obconically truncate, 22–70 × 2–3 µm; hilum inconspicuous, unthickened and not darkened.

SPECIMEN EXAMINED: IRAN, GOLESTAN PROVINCE, Shastkola forest, on *Eucalyptus camaldulensis* Dehnh., 8 Nov. 2010, M. Pirnia & R. Zare (IRAN 15481 F).

NOTE—Kobayashi introduced *Cercospora paraguayensis* on *Eucalyptus* sp. from Paraguay (see Crous 1998).

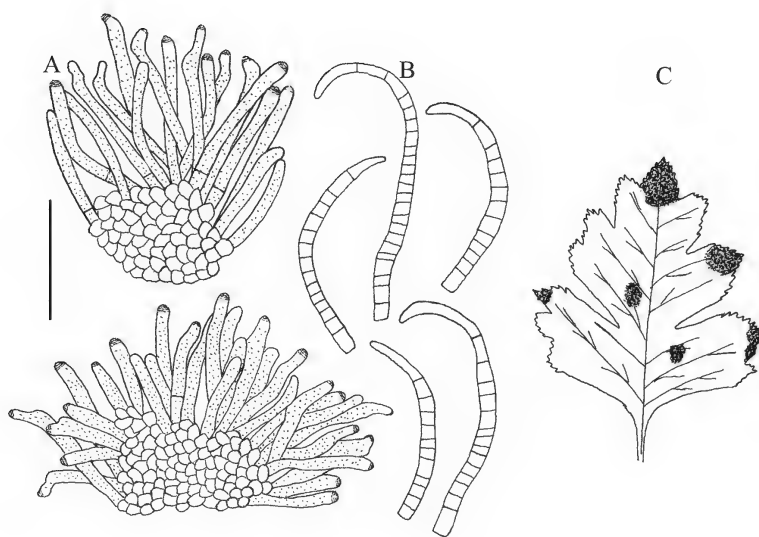


FIG. 7. *Scolecostigmina confluens* on *Crataegus pseudomelanocarpa*.
A. Conidiophores, B. Conidia, C. Symptoms on leaf (Bar = 50 µm).

Scolecostigmina confluens (Lieneman) U. Braun, New Zealand J. Bot. 37: 325 (1999)

FIG. 7

Leaf spots irregular, dark brown, scattered on leaf surface, but mostly at the tip and edge of leaves, 5–15 mm in diameter; caespituli amphigenous, mostly epiphyllous, punctiform; stromata prominent, subglobose, dark brown, 30–65 µm wide; conidiophores in dense fascicles, 15–30 stalks, arising from stromata, dark brown, paler and attenuated towards the apex, 0–1-septate, cylindrical, straight to curved, sinuous to rarely once geniculate in the upper part, not branched, verruculose, thick-walled, $25\text{--}75 \times 4\text{--}7$ µm; conidiogenous cells integrated, terminal, proliferation percurrent, annellate; conidia solitary, dark brown, paler and narrower towards the tip, subcylindrical-obclavate, slightly curved, verruculose, thick-walled, transversely pluriseptate, occasionally with few longitudinal or oblique septa, base truncate, tip subacute, $75\text{--}140 \times 4\text{--}8$ µm.

SPECIMENS EXAMINED: IRAN, GOLESTAN PROVINCE, Ghorogh forest park, on *Crataegus pseudomelanocarpa* Popov ex Lincz. 14 Sep. 2010, M. Pirnia (IRAN 15472 F); GUILAN PROVINCE, Rasht, Saravan, on *C. melanocarpa* M. Bieb., 27 July 2007, A. Khodaparast (IRAN 15473 F).

NOTE—The genus *Scolecostigmina* was originally described as having thick-walled verruculose conidiophores with conspicuous annellations and conidia

that are subcylindrical-obclavate, smooth to verruculose, transversely multi-euseptate conidia, and occasionally with a few longitudinal septa (Braun et al. 1999).

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***Phaeocollybia nigripes* (Agaricomycetes), a new species from Brazil**VICTOR R.M. COIMBRA^{1*}, TATIANA B. GIBERTONI¹ & FELIPE WARTCHOW²¹ Universidade Federal de Pernambuco, Departamento de Micologia/CCB,

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ABSTRACT — *Phaeocollybia nigripes* is described from an Atlantic Forest fragment in Pernambuco, Northeast Brazil. It is characterized by a stipe that is uniformly slender with a slightly swollen base and dark grayish brown below to almost black at the apex, brown pileus, large basidiospores that average $12.4 \times 6.6 \mu\text{m}$, hyphoid to narrowly clavate cheilocystidia, and abundant clamp connections. The holotype of *P. megalospora* var. *megalospora*, the putatively most similar taxon, was examined. A key to the six taxa reported from Brazil is also presented.

KEY WORDS — Agaricales, Hymenogastraceae, neotropic, taxonomy

Introduction

Phaeocollybia R. Heim was originally described to accommodate taxa among the brown-spored Agaricales with smooth and moist to viscid pilei and long pseudorhizas (Heim 1931). Matheny et al. (2006), based on multilocus phylogenetic analyses, placed the genus as represented by *P. festiva* (Fr.) R. Heim in the Hymenogastraceae clade, but Kirk et al. (2008), continued to refer *Phaeocollybia* to Cortinariaceae. More recently, Petersen et al. (2010), also using molecular data, treated *P. festiva* and *P. dissiliens* A.H. Sm. & Trappe in the bolbitioid clade with *Bolbitius* Fr., *Panaeolus* (Fr.) Quél., and *Conocybe* Fayod.

Phaeocollybia has been rarely reported in Brazil. The few taxa known from this country were all reported from the Amazon region by Singer (1987), who cited *P. brasiliensis* I.A. Aguiar ex Singer, *P. elaeophylla* Singer, *P. flava* I.A. Aguiar ex Singer, *P. megalospora* I.A. Aguiar ex Singer var. *megalospora*, and *P. megalospora* var. *tetraspora* Singer.

In this paper, we describe a new species based on a collection from the Atlantic Forest of Pernambuco, Northeast Brazil, and provide a key to the Brazilian *Phaeocollybia* species.

Materials & methods

One basidioma was collected in an Atlantic Forest fragment called Ecological Reserve of Dois Irmãos (8°01'15"S 34°52'30"W), a 387.4 ha area comprising a remnant of Atlantic Forest and a public zoo in the urban perimeter of the municipality of Recife (Ribeiro et al. 2007, Souza et al. 2009).

Colors are coded according to Watling (1969). The key to species is based mainly on data given by Singer (1987). Basidiospore data terminology follows Tulloss et al. (1992) but based on 25 basidiospores measured from a single basidioma (Wartchow 2009). Abbreviations include L(W) = basidiospore length (width) average, Q = the length : width ratio range as determined from all measured basidiospores, and $\bar{Q}$ = the Q value averaged from all basidiospores measured.

Scanning electron microscopy (SEM) studies were conducted at the 'Laboratório de Microscopia Eletrônica' (DF/UFPE). Sections were removed from dried basidiomata and mounted directly on aluminum stubs using carbon adhesive tabs. The fragments were coated with 8–13 nm of gold using a sputter coater and examined with a scanning electron microscope.

Phaeocollybia nigripes Wartchow & V. Coimbra sp. nov.

FIGS. 1-6, 8

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Ab Phaeocollybia megalospora var. *megalospora* differt stipe coloribus apice niger versus fuscus basin, cheilocystidiis tenui clavati vel cylindraceous et catenatus, et pileipellis hipodermio ex elementis pro ratione voluminis.

HOLOTYPE: Brazil, Pernambuco, Recife, Ecological Reserve of Dois Irmãos, 10.iii.2009 V.R.M. Coimbra, J.M. Baltazar & L. Trierveiler-Pereira s.n. (URM 82277, **holotype**).

ETYMOLOGY: Latin for 'black foot', in reference to the stipe color.

PILEUS 11 × 10 mm, conic, reddish brown to dark brown ('Date brown 24'), surface smooth, margin slightly short-sulcate and involute. LAMELLAE adnexed, close, lamellulae present, dark grayish brown (slightly lighter than 'Drab 33') with dark gray edges (under 10× lens), edges entire. Veil not evident. STIPE 70 × 1.5–2 mm (upper portion apart from pseudorhiza 55 mm long), cylindrical above a swollen base, central, dark brown ('Cigar brown 16'), shiny metallic graphite-black at apex with a vinaceous base under intense light, surface smooth, hollow. Pseudorhiza thin, spiraling, ¼ of the stipe length.

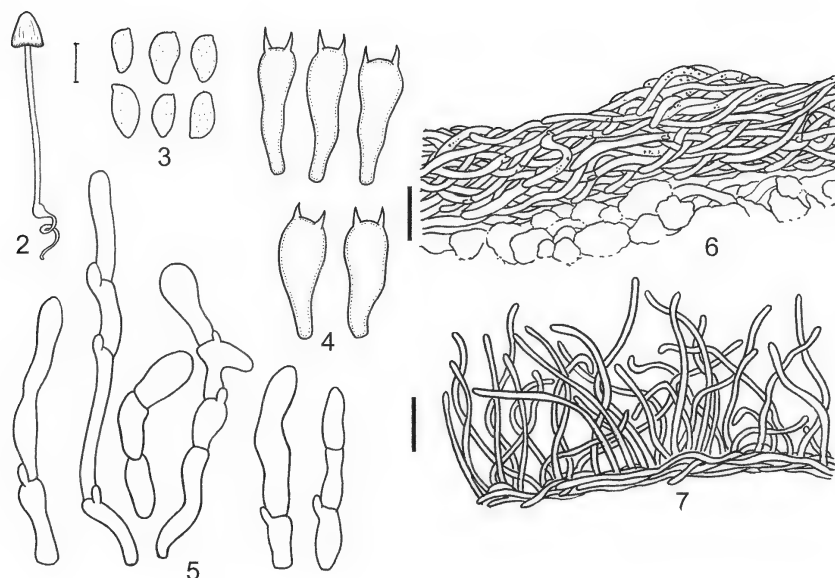
BASIDIOSPORES (10–)10.8–14.5(–15) × (5.5–)6–7.3(–8) µm (L = 12.4 µm; W = 6.6 µm; Q = (1.69–)1.77–2.00(–2.29); $\bar{Q}$ = 1.88), amygdaliform to sometimes limoniform, thin-walled, surface minutely verrucose, brown. BASIDIA 24–33 × 9–11 µm, clavate, thin-walled, with prominent sterigmata, predominantly 2-sterigmate. PLEUROCYSTIDIA absent. CHEILOCYSTIDIA (13–)16–25 × 5–6 µm, narrowly clavate to hyphoid (located in terminal position of chains of short celled hyphae), forming dense tufts on the sterile edge, thin-walled, hyaline. TIBIFORM DIVERTICULA abundant on pseudorrhizal pellis, 11–32 × 1.8–3 µm, narrowly lanceolate to subcapitate, sometimes with apical droplet, some with small lateral projections, hyaline, colorless, thin walled; on stipe more scattered



FIG. 1. *Phaeocollybia nigripes* (holotype). Basidioma. Scale bar = 10 mm.

and slightly smaller $11\text{--}16 \times 2\text{--}3 \mu\text{m}$, hyaline, thin walled. PILEIPELLIS with periclinal to sometimes anticlinal hyphae of $3\text{--}6 \mu\text{m}$ wide, pigments encrusting, yellowish brown, showing some gelatinized zones; hypodermium somewhat pseudoparenchymatous, elements $20\text{--}27 \times 13\text{--}18 \mu\text{m}$, yellowish-brown. LAMELLAR TRAMA regular and parallel, hyphae ranging $4.5\text{--}15.5 \mu\text{m}$, thin-walled. CLAMP CONNECTIONS abundant.

ECOLOGY & DISTRIBUTION: Solitary on soil under dicotyledonous trees, including representatives of the ectomycorrhizal families (Smith & Read 2008) *Caesalpinaceae* [*Senna* Mill., *Chamaecrista* (L.) Moench, *Dialium* L., *Hymenaea* L., *Sclerolobium* Vogel, *Zollernia* Maximil. & Nees], *Nyctaginaceae* (*Boerhavia*



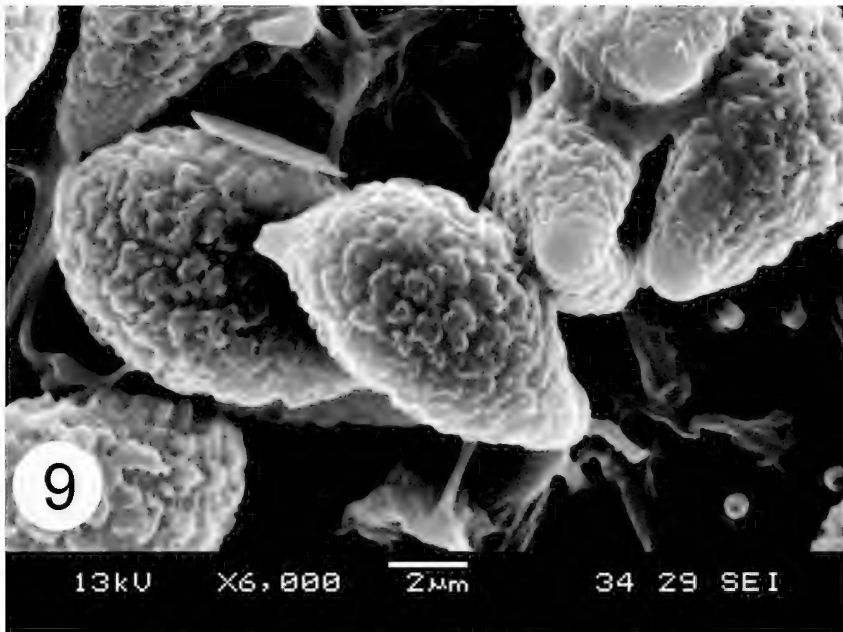
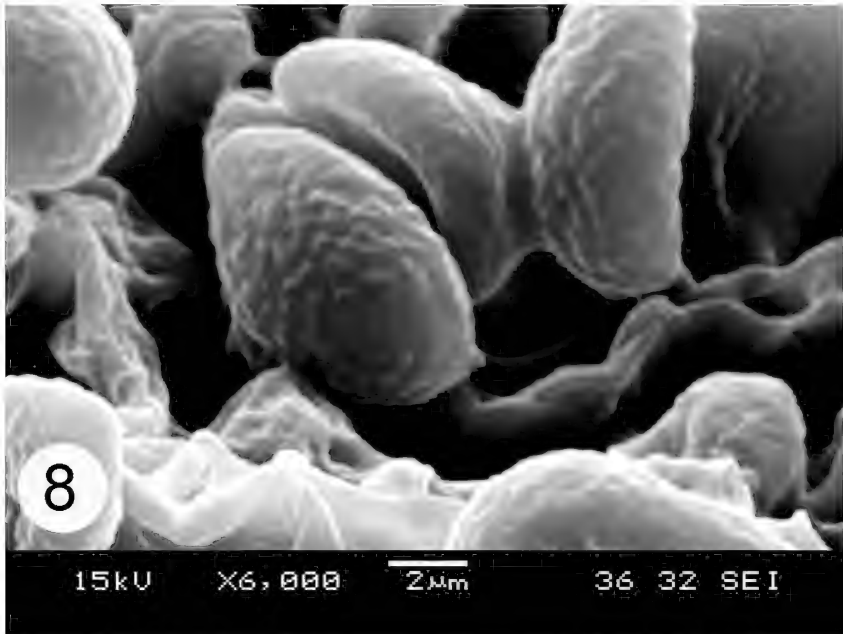
FIGS. 2–6. *Phaeocollybia nigripes* (holotype): 2. Basidioma. 3. Basidiospores. 4. Basidia. 5. Hyphal elements of the lamella edge. 6. Pileipellis and adjacent inflated elements of the hypodermium. FIG. 7. *P. megalospora* var. *megalospora* (holotype): Pileipellis and adjacent hyphae of the hypodermium. Scale bars: 2 = 10 mm; 3–7 = 10 μ m.

L., *Guapira* Aubl.) and *Polygonaceae* (*Coccoloba* P. Browne). Known only from the type locality in the Atlantic Forest of Northeast Brazil.

ADDITIONAL SPECIMEN EXAMINED: *Phaeocollybia megalospora* var. *megalospora*—BRAZIL. Amazonas, road Manaus–Caracará km 45, 11.vii.1977, R. Singer & I. Araujo, Singer B 10082 (INPA 102697, holotype!).

REMARKS: The distinctive microscopic features of *P. nigripes* include basidiospores longer than 11 μ m, predominantly 2-spored basidia, cylindric-hyphoid to narrowly clavate cheilocystidia, and a slightly pseudoparenchymatous hypodermium. The pseudorhiza of our new species was broken during collection.

Following the classification proposed by Bandala & Montoya (1994), *P. nigripes* would be referred to subgen. *Fibulophaeocollybia* Bandala & Montoya sect. *Subattenuatae* Singer based on the presence of clamp connections (most easily seen at the cheilocystidial bases) and limoniform basidiospores longer than 7 μ m (Bandala & Montoya 1994). Norvell & Exeter (2009), who felt that infrageneric classification is premature in the absence of molecular analyses of the whole genus, suggested that cheilocystidial morphology might be a better



FIGS. 8–9. SEM of basidiospores (photos by Sérgio Santos).
8. *Phaeocollybia nigripes*. 9. *P. megalospora*.

morphological character for identifying monophyletic groups below genus level in *Phaeocollybia*.

Phaeocollybia megalospora var. *megalospora*, characterized by Singer (1987) as also producing 2-sterigmate basidia and similar sized basidiospores, differs in its yellowish to brownish stipe, pileipellis with a gelatinized ixocutis and cutiform rather than pseudoparenchymatous hypodermium, ampullaceous cheilocystidia, and more strongly verrucose basidiospores (Singer 1987). We examined the holotype (Figs. 7, 9) of *P. megalospora*, which we believe is the same basidioma depicted in Singer (1987: Plate III, fig. 5), and noted that the stipe was apparently broken and lacked the pseudorhiza (which typically bears abundant tibiiform diverticula according to Norvell 1998), so that only one very small ($<7 \times 2 \mu\text{m}$) tibiiform diverticulum was observed. The pileipellis comprised gelatinized erect, non-incrusted, $1\text{--}3.5 \mu\text{m}$ wide, yellowish hyphae with the hypodermium comprising hyphae $\leq 5 \mu\text{m}$ wide, radially oriented, and with dark brown vacuolar pigments (Fig. 7). The basidiospores are very similar in size $11\text{--}15 \times (6\text{--})6.5\text{--}8 \mu\text{m}$ ($L = 12.9 \mu\text{m}$; $W = 7.2 \mu\text{m}$; $Q = (1.43\text{--})1.50\text{--}2.00(-2.14)$; $Q = 1.80$) but more strongly verrucose (Fig. 9) than those found in our species, supporting our conclusion that *P. megalospora* var. *megalospora* and *P. nigripes* are distinct species.

Another taxon with blackish tints in the basidioma is *P. singularis* E. Horak & Halling from Colombia (Horak & Halling 1991) and the Talamanca Mountains in Costa Rica (Norvell pers. corr.), but it obviously differs in the blackish lavender pileus, violaceous stipe, smaller ($8\text{--}9.5 \times 4.5\text{--}5 \mu\text{m}$) basidiospores, and tibiiform cheilocystidia.

Phaeocollybia phaeogaleroides Norvell is another similar species with relatively large basidiospores. It shares with *P. nigripes* the dark brown pileus and stipe, clamp connections, and cylindrical cheilocystidia (Norvell 2002) but differs in the hygrophanous pileus, shorter narrower basidiospores $9\text{--}12(-13) \times 5\text{--}6.5 \mu\text{m}$ ($L = 10 \mu\text{m}$; $W = 5.8 \mu\text{m}$), 4-spored basidia, and longer ($\leq 80 \mu\text{m}$) subcapitate cheilocystidia (Norvell 2002).

Phaeocollybia longistipitata Halling & E. Horak, *P. odorata* E. Horak, *P. pleurocystidiata* Norvell & Redhead, *P. singeri* Guzmán et al., and *P. tentaculata* E. Horak are other slender stiped taxa that are segregated primarily by their smaller basidiospores and lack of clamp connections (Horak 1977, Bandala et al. 1989, Norvell & Redhead 2000, Halling & Horak 2008).

Phaeocollybia columbiana Singer from tropical rain forest of Colombia also shares relatively large ($10\text{--}12 \times 6\text{--}7 \mu\text{m}$) basidiospores. However, it differs in the spadiceous pileus with whitish centre, ochraceous brown stipe, ventricose cheilocystidia, and absence of clamp connections (Singer 1970, Horak 1977).

Phaeocollybia amazonica Singer, from the Bolivian Amazon, differs from *P. nigripes* macroscopically in pileus size ($40\text{--}45 \text{ mm}$ diam), ochraceous brown

to rusty lamellae, and cinnamon stipe and microscopically in basidiospore shape and larger ($>30 \times 6 \mu\text{m}$) cheilocystidia (Singer 1961, 1970).

Other lowland Amazonian Brazilian taxa — *P. brasiliensis*, *P. elaeophylla*, *P. flava*, *P. megalospora* var. *tetraspora* — are easily segregated by their smaller basidiospores (Singer 1987).

Here we also address the ecological status of *Phaeocollybia nigripes*. Earlier studies implied *Phaeocollybia* as saprotrophic with the pseudorhizae arising from buried wood (Smith 1957; Smith & Trappe 1972). Subsequently Redhead & Malloch (1986), who studied the temperate *P. christinae* (Fr.) R. Heim and *P. jennyae* (P. Karst.) Romagn., concluded that *Phaeocollybia* is parasitic based on the successful excavation of a pseudorhiza connected to a senescent spruce rootlet. Norvell (1998), demonstrating that *P. redheadii* Norvell, *P. kauffmanii* (A.H. Sm.) Singer, and several other taxa present ectomycorrhizal mantles and Hartig-net in western hemlock and noble fir roots, emphasized that all Pacific Northwest North American *Phaeocollybia* species are found in forests with *Tsuga*, *Picea*, *Abies*, *Pseudotsuga*, *Lithocarpus*, *Quercus*, and/or *Pinus*, all ectomycorrhizal-forming genera (Smith & Read 2008). Most recently, Norvell & Exeter (2009) cite the presence of Hartig-net and high nitrogen isotope readings presented by the studied species as the main characters supporting the ectomycorrhizal nature of *Phaeocollybia* in the Pacific Northwest.

Neotropical phaeocollybias from Colombia and Costa Rica occur mostly under the ectomycorrhizal tree genus *Quercus* (Singer 1987, Horak & Halling 1991, Halling & Horak 2008), while others from lowland Amazon forests were found in “terra firme,” a typical anectotrophic forest type according to Singer & Araújo (1979). In the case of *P. nigripes*, although no ectomycorrhizal connection was detected, representatives of *Caesalpinaceae*, *Nyctaginaceae*, and *Polygonaceae* occurred in the collection area (Guedes 1998, Ferreira et al. 2007, Souza et al. 2009); these families include genera shown to have ectomycorrhizal associations in the tropical forests of Ecuador: e.g., *Coccoloba* (*Polygonaceae*) and *Guapira* (*Nyctaginaceae*) (Tedersoo et al. 2010).

Artificial key for the Brazilian taxa of *Phaeocollybia*

1. Basidiospores small, $5\text{--}5.5 \times 4.5\text{--}5 \mu\text{m}$; pileus yellow *P. flava*
- 1'. Basidiospores longer, $> 7 \mu\text{m}$ long; pileus with brown tints 2
2. Basidiospores $\leq 10\text{--}15 \mu\text{m}$ long; basidia mostly with two sterigmata 3
- 2'. Basidiospores with largest length not longer than $11.5 \mu\text{m}$; basidia with four sterigmata (in *P. elaeophylla* 1–4 sterigmata) 4
3. Stipe yellowish to brownish towards the base; cheilocystidia ampullaceous; hypodermium cutiform made of hyphae $3\text{--}9 \mu\text{m}$ wide
..... *P. megalospora* var. *megalospora*

- 3'. Stipe dark brown with shiny metallic graphite-black apex; cheilocystidia narrowly clavate to hyphoid (located in terminal position of chains of short-celled hyphae); hypodermium pseudoparenchymatous made of elements of $20\text{--}27 \times 13\text{--}18 \mu\text{m}$ *P. nigripes*
4. Lamellae olive; basidiospores $8.5\text{--}10.3 \times 4.5\text{--}6.5 \mu\text{m}$, cheilocystidia heteromorphous $17\text{--}49 \times 3.5\text{--}5.5 \mu\text{m}$, sometimes tibiiform-like *P. elaeophylla*
- 4'. Lamellae with no olive color 5
5. Pileus striate; basidiospores $(7\text{--})7.5\text{--}8.3 \times 4.7\text{--}5.5 \mu\text{m}$; cheilocystidia ventricose frequently capitate (tibiiform?) $25\text{--}32 \times 6\text{--}7.5 \mu\text{m}$ *P. brasiliensis*
- 5'. Pileus not striate; basidiospores $9.5\text{--}11.5 \times 6\text{--}7 \mu\text{m}$, cheilocystidia clavate, cylindrical or utriform of $40\text{--}50 \times 3.8\text{--}6.5 \mu\text{m}$ *P. megalospora* var. *tetraspora*

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***Peziza michelii* and its ectomycorrhizae with *Alnus nitida* (Betulaceae) from Pakistan**T. ASHRAF^{1*}, M. HANIF² & A. N. KHALID³

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ABSTRACT — *Peziza michelii* and its ectomycorrhizae with *Alnus nitida* are characterized morphologically and molecularly by nrDNA ITS1-5.8S-ITS2 sequence analyses. Both the fungus and its morphotype are new records for Pakistan.

KEY WORDS — operculate asci, *Pezizales*, phylogeny

Introduction

Peziza Dill. ex Fr. (*Pezizales*, *Pezizaceae*) is a large, widely distributed heterogeneous genus represented by 104 species (Kirk et al. 2008). Its trophic status ranges from saprobic to mycorrhizal (Hansen et al. 2001).

Identification of pezizalean mycorrhizae using morphological or conventional methods has remained difficult since they lack hyphal strands, connecting apothecium, and diagnostic morphotypes. Generally pezizalean ECM (ectomycorrhizae) possess a thin pseudoparenchymatous mantle, infrequent emanating hyphae, and rhizomorphs, and clamp connections are lacking (Agerer 1991, 2001). At the present time molecular tools are quite helpful for ECM identification, and the ECM of many *Peziza* species have been confirmed using such tools. Species identified as mycorrhizal with deciduous trees include *Peziza depressa* Pers., *P. michelii*, *P. ostracoderma* Korf, *P. succosa* Berk., and *P. succosella* (Le Gal & Romagn.) M.M. Moser ex Aviz.-Hersh. & Nemlich (Tedersoo et al. 2006).

Betulaceae is represented in Pakistan by two genera, *Alnus* and *Betula* (Nair 1975), for which there are no previous reports about ectomycorrhizal status from Pakistan. Previously, *P. michelii* has been reported as ectomycorrhizal with *Alnus* sp., *Picea abies* (Tedersoo et al. 2009), *Betula* sp. (Tedersoo et al.

2008), *Fagus orientalis* (Bahram et al. 2012) *Tsuga canadensis* (McLenon-Porter 2008), and *Tilia* sp. (Lang et al. 2011). Mycorrhizal associations of *Alnus* with *P. michelii* are confirmed molecularly in this study.

Ahmad et al. (1997) and Ashraf & Khalid (2012) have reported 89 pezizalean taxa (including nine *Peziza* species) from Pakistan, and ascocarp surveys in Pakistan account for eight known to be ectomycorrhizal. Molecular characterization of *Pezizales* and their ectomycorrhizae in Pakistan is in progress. Our study, which documents association of *P. michelii* with *Alnus nitida* (Spach) Endl. in Asia for the first time, is the first attempt at morphological and molecular characterization of ascomycete morphotypes from Pakistan.

Materials & methods

Ascomata were collected and photographed; necessary data was recorded in field and specimens were dried with the help of a fan heater. Ascomata were rehydrated and free hand sections were made in the laboratory. Microscopic (morpho-anatomical) characters were noted at 10× and 40× and drawings were made with the help of camera lucida.

Soil under *A. nitida* was sampled and washed to isolate, characterize, and identify ECM. The selected morphotypes were characterized morphologically following Agerer (2001) and have been deposited in Herbarium of Botany Department (LAH), University of the Punjab in Lahore.

DNA was extracted from dried ascomata and morphotypes using Extract N. Amp.™ Plant kit (SIGMA). The nrDNA ITS1–5.8S–ITS2 region was amplified using fungal specific primers pairs (ITS1F/ITS4) following manufacturer's protocol with denaturation at 94°C for 4 min, followed by 35 cycles of 45 sec at 94°C, 45 sec at 54°C, and 1 min 30 sec at 72°C, and a final extension at 72°C for 2 min. After purification, the PCR products underwent bidirectional sequencing by Macrogen (South Korea). GenBank ITS sequences were BLAST-searched for sequence comparison and identification. Selected sequences were aligned using Clustal W and corrected manually. All ambiguous insertions and deletions were removed prior to further analyses. Sequences have been accessioned in GenBank.

Results

Alnus nitida roots were found to be colonized by *Peziza michelii*. The BLAST search of the ITS sequences from *P. michelii* ascomata and morphotypes associated with *Alnus nitida* obtained in the present study showed a 99% identity and 98% query coverage match with *P. michelii* from Denmark (DQ200839.1). The identity percentage was calculated at 96.3, showing a 97% identity and minimum divergence of 1.5 and 0.8 compared with *Peziza michelii* (DQ200838.1 and DQ200839.1) respectively (PLATE 1). The topology and identification of *P. michelii* was confirmed by the neighbor-joining method

PLATE 2. Phylogenetic tree based on ITS1-5.8S-ITS2 nucleotide sequences adjusted to 722 bases and constructed by the neighbor-joining method using MegAlign. The scale indicates the percentage of base difference (% divergence). Sequence data for phylogenetic analysis were taken from the GenBank nucleotide sequence database for *Peziza*, *Cazia*, *Chromelosporium*, and *Mycoclelandia*.

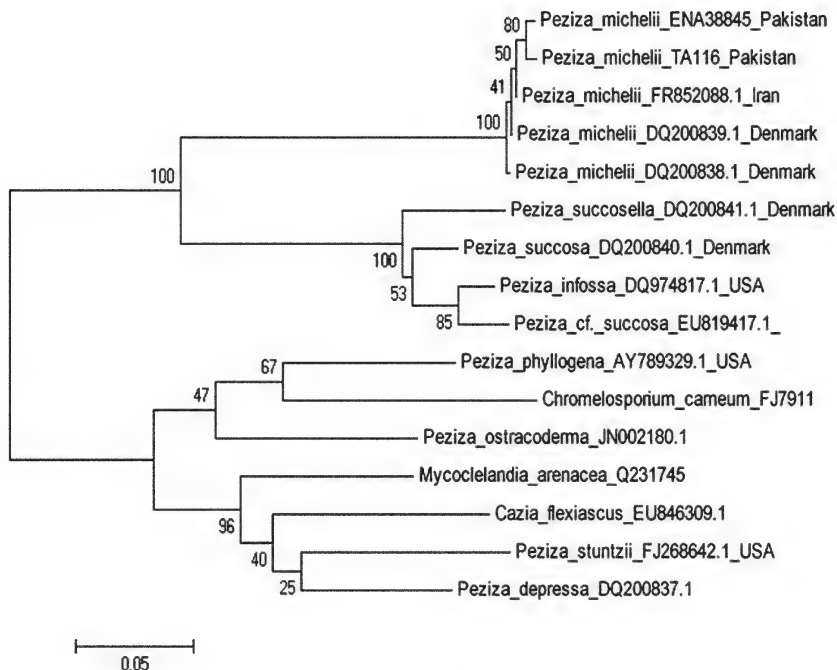


PLATE 3. Molecular Phylogenetic analysis was inferred by using the Maximum Likelihood method. The tree with the highest log likelihood (-6466.0969) is shown. Bootstrap values are shown against each branch. Initial tree(s) for the heuristic search were obtained automatically: maximum parsimony method was used when common sites numbered <100 (or <1/4 total sites); otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in number of substitutions per site. The analysis involved 16 sequences.

Taxonomy

Peziza michelii (Boud.) Dennis, Brit. Cup Fungi & Allies: 15 (1960)

PLATE 4

APOTHECIA cupulate, medium-sized, diam. up to 5 cm, deeply concave, margins entire, but turning inwards, somewhat raised and torn, hymenium reddish brown with purplish tinge, smooth, outer surface yellow to yellowish brown, sessile to subsessile, solitary. HYMENIUM 290 μ m thick. ASCI cylindrical, operculate, unitunicate, 8-spored, uniseriate, strongly amyloid at apex, but diffusely blue along length, slightly narrowing near base, 260–278(–300) $\times$ 13–17 μ m. ASCOSPORES uniseriate, ellipsoid, biguttulate, ornamented, 15–17(–19) $\times$ 9–10 μ m, ornamentation verrucose to warted: which appear elongated, irregularly and widely spaced, 0.3–0.7 μ m high. PARAPHYSES long, slender, septate, with light brownish content, straight, 2–3 μ m wide, slightly thick up to 5–6 μ m at tip. EXCIPULUM: Ectal excipulum a textura globulosa to angularis, 100–160 μ m thick, cells hyaline to slightly brown, thin-walled, diam. 10–22

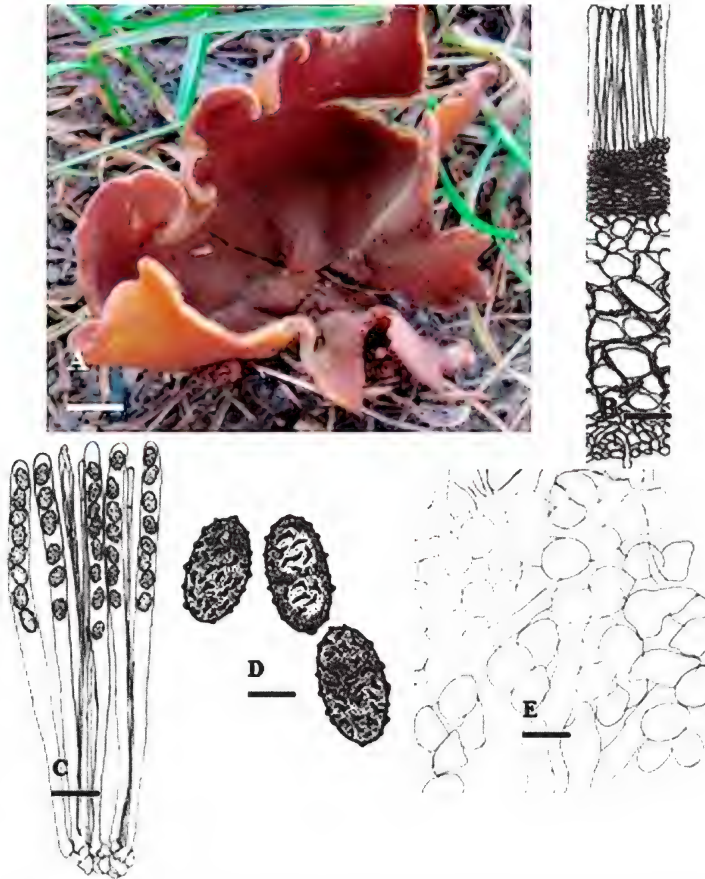


PLATE 4. *Peziza michelii*: A. Apothecium. B. Apothecial section showing hymenium, sub-hymenium, medullary, and ectal excipulum. C. Part of hymenium, showing asci with ascospores and paraphyses. D. Ascospores. E. Excipulum. Scale bars: A = 0.5 cm; B = 90 μ m; C = 30 μ m; D = 6 μ m; E = 80 μ m

μ m; medullary excipulum a textura globulosa, 500 μ m thick, larger sub-globose thin-walled cells, 22–40 μ m diam., interspersed with numerous interwoven delicate hyphae $\leq$ 8 μ m diam.

MATERIAL EXAMINED: PAKISTAN: KHYBER PAKHTUNKHWA, Khanspur, Helipad, Himalayan moist temperate forests, 2575 m (8205 ft) altitude, solitary, on ground, mossy substrate, 21 Aug. 2010, T. Ashraf TA-116 (LAH 210810; GenBank JN836748).

Morphological description

PLATE 5

ECTOMYCORRHIZAL SYSTEM simple, axis 4–5 $\times$ 1.0–1.3 mm. UNRAMIFIED ENDS rounded to bent, bifurcate, 2–3 mm long and 0.8–1.2 mm in diam.,

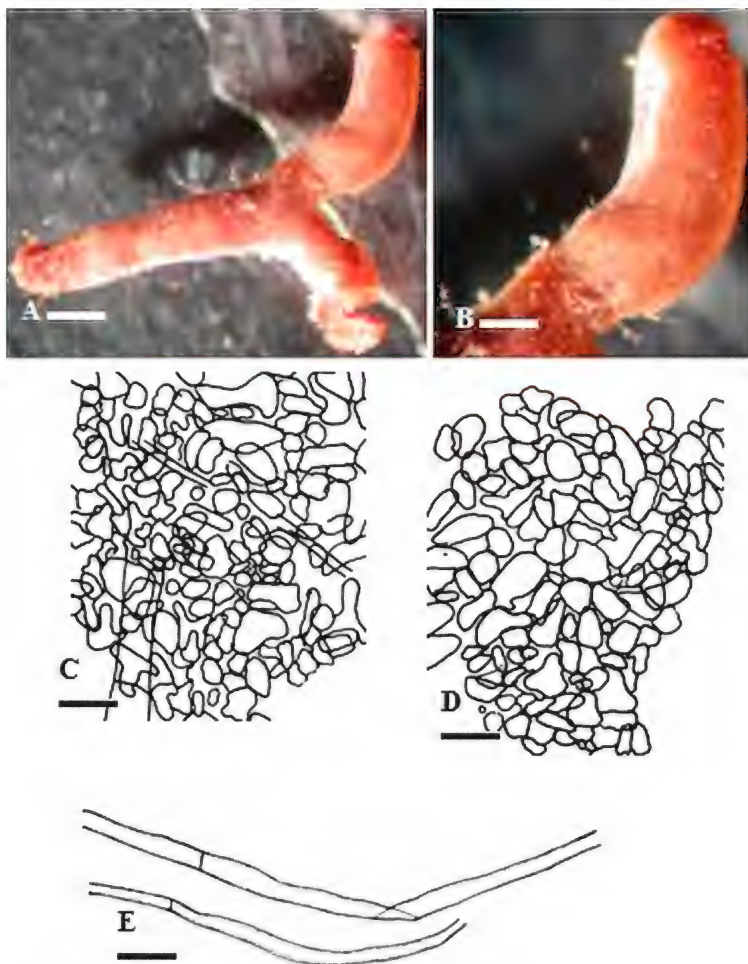


PLATE 5. ECM of *Peziza michelii*: A-B. Habit. C. Pseudoparenchymatous outer mantle. D. Pseudoparenchymatous inner mantle. E. Emanating hyphae. Scale bars: A=0.5 mm; B= 0.5 mm; C-D = 2 μ m; E= 1.5 μ m.

younger tips creamy white, older tips brown to black. Texture of system felty to velvety with matte luster, host tissue not visible under the sheath. RHIZOMORPH absent. EMANATING HYPHAE rare, straight.

MANTLE pseudoparenchymatous in all layers, OUTER MANTLE pseudoparenchymatous with irregular (ovoid to epidermoid) cells, hyphal cells infrequent and inconspicuous, hyphae without clamps, light yellowish, no cell

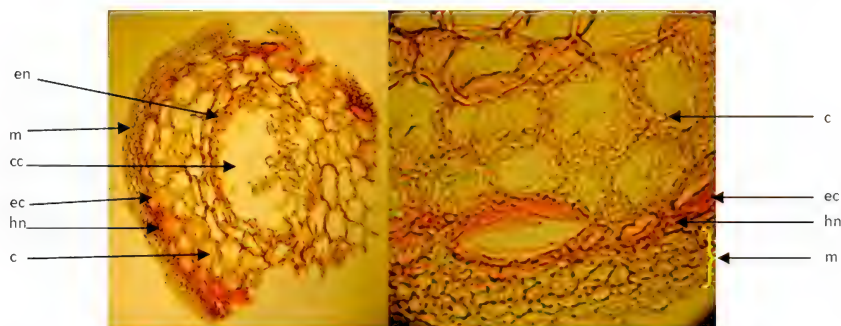


PLATE 6. Cross section of *Alnus nitida* ectomycorrhizal root: m = mantle, ec = ectoderm, c = cortex, en = endoderm, cc = central cylinder, hn = hartig net.

contents visible, cells $14-15 \times 10-14 \mu\text{m}$. INNER MANTLE pseudoparenchymatous, cells colorless to light yellowish, cells roundish to irregular in shape, no matrix material observed, cells $13-15 \times 11-14 \mu\text{m}$.

MANTLE THICKNESS $36 \mu\text{m}$, ≤ 5 layers thick, discernible, cells rectangular to tangentially oval and/or tangentially elliptical. HARTIG NET paraepidermal, followed by large cortical cells, endodermis and central cylinder (PLATE 6)

MATERIAL EXAMINED: PAKISTAN: KHYBER PAKHTUNKHWA, Khanspur, Helipad, Himalayan moist temperate forests, 2575 m (8205ft) altitude, roots of *Alnus nitida*, 15 May 2010, M Hanif ENA38845 (LAH 150509; GenBank JN836749).

Discussion

The present work is the first report of *Peziza michelii* from Pakistan. This species can be found frequently in Himalayan moist temperate forests of Pakistan. Phylogenetically, *P. michelii* clusters with the similar species from USA, Iran, and Denmark.

Tedersoo et al. (2006) studied the morphotypes of *P. michelii* morphologically and used molecular markers to identify the mycobiont. They found these ectomycorrhizae to be solitary, rare, in small clusters, minute, conspicuous, and yellow green to olive green. This finding differed from the present report and morphotypes associated with *Alnus nitida* from Pakistan. Those morphotypes we studied are simple, creamy white with brown to black older tips, and lacking a rhizomorph. The anatomical features of the mantle surface also differ slightly in both morphotypes, with a mantle surface varying from smooth to finely granular in the morphotype reported by Tedersoo et al. (2006) versus the *A. nitida* morphotype, which is felty to velvety with matte luster with almost equal thickness (2–4 hyphal layers vs. 5 hyphal layers). Other mantle features in both studies also varied. The morpho-anatomic features of both morphotypes

of *Peziza michelii* may vary because of interaction of mycobiont with different photobionts.

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***Erysiphe javanica* sp. nov.,
a new tropical powdery mildew from Indonesia**JAMJAN MEEBOON¹, IMAN HIDAYAT² & SUSUMU TAKAMATSU^{1*}¹Department of Bioresources, Graduate School, Mie University, Tsu 514-8507, Japan²Microbiology Division, Research Center for Biology, Indonesian Institute of Sciences,
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ABSTRACT — During the collection of tropical powdery mildews in Cibodas Botanical Garden and Mount Tangkuban Perahu in Indonesia, we found interesting specimens on *Castanopsis javanica* (Fagaceae). Phylogenetic analysis using a combination of 28S and ITS rDNA sequences clearly showed that this fungus forms a distinct lineage among the *Erysiphe* species found on hosts of the plant genus *Castanopsis*. Therefore, we consider this fungus a new species of *Erysiphe*, described here as *Erysiphe javanica*. Differences between *E. javanica* and closely related *Erysiphe* species are discussed.

KEY WORDS — Asia, *Brasiliomyces*, *Erysiphaceae*, phytopathogenic fungi, tropic

Introduction

Species of *Erysiphe* R. Hedw. ex DC. on fagaceous plants have been reported as a unique group of powdery mildews because they comprise not only fungi with simple mycelioid appendages that are characteristic for *Erysiphe* sect. *Erysiphe* (Braun & Takamatsu 2000), but also encompass taxa with different types of chasmothecial appendages previously belonging to the genera *Brasiliomyces* Viégas [rudimentary/mycelioid], *Uncinula* Lév. [uncinuloid], and *Microsphaera* Lév. [dichotomously branched at the apex] (Braun & Takamatsu 2000, Takamatsu et al. 2007, Divarangkoon et al. 2011). In addition, Divarangkoon et al. (2011) reported that the lineage of fagaceous *Erysiphe* spp. consists of not only taxa with multiple peridial cell layers but also species characterised by a single peridial cell layer previously assigned to *Brasiliomyces* [i.e., *E. trina* Harkn. (= *Brasiliomyces trinus* (Harkn.) R.Y. Zheng), *E. asiatica* Meeboon et al., and *E. monopერიდაта* Meeboon et al. (Divarangkoon et al. 2011)]. These results suggest that different appendage types and single-layered peridial cells evolved on fagaceous hosts.

Several authors (Zheng 1984, Braun 1987, To-anun et al. 2003) have discussed the significance of the peridial cells in separating *Brasiliomyces* from *Erysiphe*. Zheng (1984) noted that *Brasiliomyces* must be treated as an independent genus due to having a single layer of peridial cells not differentiated into inner and outer layers as described in the genus *Cystotheca* Berk. & M.A. Curtis. This argument was supported by Braun (1987) based on the reexamination of the type species, *B. malvastri* Viégas. Before 2011, eight *Brasiliomyces* species had been described based on this morphological criterion (To-anun et al. 2003; Divarangkoon et al. 2011), of which there were recorded on fagaceous host plants — *B. trinus* (= *E. trina*), *B. cyclobalanopsidis* K.C. Kuo et al. (= *E. cyclobalanopsidis* (K.C. Kuo et al.) U. Braun), *B. kumaonensis* N. Ahmad et al. (= *E. kumaonensis* (N. Ahmad et al.) U. Braun). Braun & Paul (2009) placed these three species on fagaceous hosts in synonymy under *Erysiphe*, because *E. trina* nested in the lineage of *Erysiphe* emend. Braun & Takamatsu (2000).

During a March 2011 visit to the Cibodas Botanical Garden and Mount Tangkuban Perahu on Java Island (Indonesia), we found specimens morphologically similar to *E. asiatica* and *E. monoperidiata*. Molecular analyses showed that the rDNA sequences of the specimens form an independent lineage separated from *E. asiatica* and *E. monoperidiata* sequences. We therefore propose this fungus as a new species.

Materials & methods

Morphological examination

Specimens were collected in two locations of West Java province (Indonesia), namely, Cibodas Botanical Garden, Bogor and Mount Tangkuban Perahu, Bandung. All collections were conducted in March 2011. Details of host name, collection date, place, and collector were recorded. Morphological examinations were carried out as outlined in Divarangkoon et al. (2011). Mycelia and chasmothecia were stripped from leaf surfaces with a clean needle, mounted on a microscope slide, and examined in 3% NaOH using a light microscope with phase contrast 10×, 20×, and 40× objectives. Thirty chasmothecia, asci, and ascospores were measured per sample. Specimens were deposited at the National Museum of Nature and Science (TNS), Japan; Mie University Mycological Herbarium (MUMH), Japan; and Herbarium Bogoriense (BO), Indonesia.

Phylogenetic analysis

Whole-cell DNA was extracted from chasmothecia using the chelex method (Walsh et al. 1991) as described in Hirata & Takamatsu (1996). The 5'-end of the 28S rDNA, including the domains D1 and D2, and ITS region, including the 5.8S rDNA, were amplified by polymerase chain reaction (PCR) and then sequenced using protocols as described in Takamatsu et al. (2006). Representative sequences determined in this study were deposited in DNA databases (DDBJ, EMBL, GenBank) under the accession numbers of JQ220151–JQ220162. Sequences generated from the rDNA ITS region and D1/D2 domains were aligned using MEGA 5 (Kumar et al. 2008) with *Erysiphe*

and *Brasiliomyces* sequences retrieved from DNA databases (DDBJ, EMBL, GenBank). The alignment was deposited in TreeBASE (<http://www.treebase.org/>) under the accession number of S12121. Maximum parsimony (MP) analysis was done in PAUP* 4.0b10 (Swofford 2002) with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985). Tree scores, including tree length, CI, RI, and RC, were also calculated.

Taxonomy

Erysiphe javanica Meeboon & S. Takam., sp. nov.

FIG. 1

MYCOBANK MB 563656

Similar to *Erysiphe asiatica*, but differing in 5–7-spored asci and occurrence on *Castanopsis javanica*.

TYPE: on *Castanopsis javanica* (Blume) A. DC. (*Fagaceae*), Indonesia, West Java province, Bogor, Cibodas Botanical Garden, 14 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (Holotype: TNS-F-44236; isotype: MUMH 5153; ex-type rDNA sequences, JQ220160 (28S), JQ220162 (ITS)).

ETYMOLOGY: the species epithet refers to the island where the specimens were collected.

COLONIES hypophyllous, persistent, forming irregular white patches on the host surfaces. HYPHAE hyaline, superficial, septate, branched, 3–6 μm wide. APPRESSORIA well-developed, coral-like, single or occasionally opposite in pairs. CONIDIOPHORES and CONIDIA unknown. CHASMOTHECIA scattered to

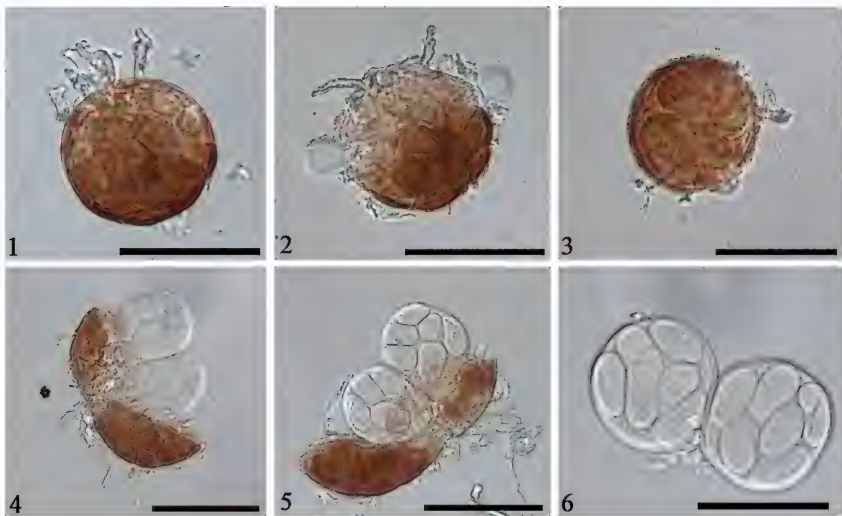


FIG. 1. *Erysiphe javanica* sp. nov.: 1–3, chasmothecia; 4–6, asci with 5–7 spores. Bars = 50 μm .

gregarious, (41–)50–66(–69) μm diam., globose, containing 2 asci; appendages present, rarely absent, poorly developed, mycelioid, (8–)17–73(–95) $\times$ (2–)3–5(–8), colourless, aseptate, thin-walled, smooth. PERIDIUM thin, one conspicuous layer, yellowish to light brown, semitransparent. Asci globose to subglobose, sessile or short-stalked, (30–)36–50(–76) $\times$ (26–)27–44(–53) μm , 5–7-spored. ASCOSPORES ellipsoid-ovoid, hyaline, (10–)11–24(–26) $\times$ (5–)8–14(–17) μm .

ECOLOGY & DISTRIBUTION: on *Castanopsis javanica*, Indonesia.

ADDITIONAL COLLECTIONS EXAMINED: on *Castanopsis javanica*: INDONESIA. WEST JAVA PROVINCE, Bogor, Cibodas Botanical Garden, 14 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (MUMH 5147, BO22660, BO22661, BO22662, BO22663); Bandung, Mount Tangkuban Perahu, 12 March 2011, J. Meeboon, I. Hidayat & S. Takamatsu (MUMH 5123, BO22655, BO22656, BO22657, BO22658, BO22659).

Phylogenetic analysis

In the 28S+ITS combination sequence data set, 1091 of 1277 total characters used in the MP analysis were constant, 58 characters were variable and parsimony-uninformative, and 128 characters were parsimony-informative. A total of 240 equally parsimonious trees (TL = 212, CI = 0.934, RI = 0.924, RC = 0.863) were generated by the MP analysis (FIG. 2). All *Erysiphe* species found on *Castanopsis* or *Lithocarpus* form a monophyletic clade with 98% bootstrap support. *Erysiphe javanica*, *E. asiatica*, and *E. monoperidiata* form independent clades with 99%, 100% and 66% bootstrap supports, respectively.

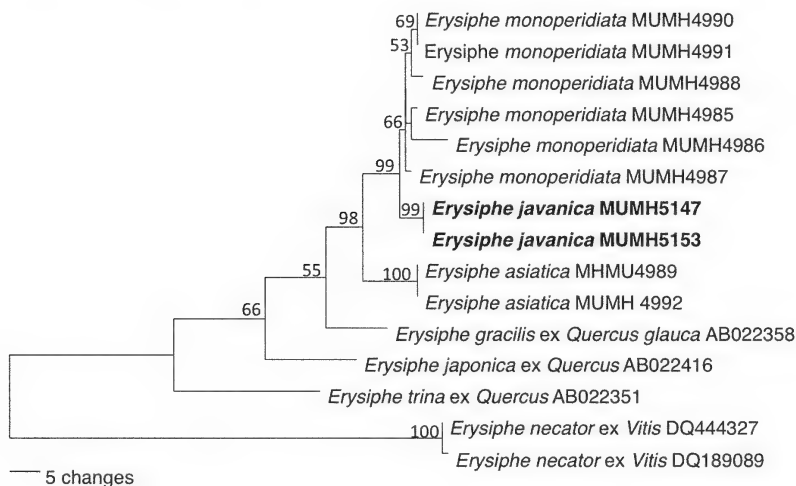


FIG. 2. Phylogeny of *Erysiphe javanica* inferred from a combination of 28S + ITS rDNA sequences using maximum parsimony (MP) method. Bootstrap support percentages (1000 replications; $\geq 50\%$) are shown on the branches.

TABLE 1. Morphological comparison of *Erysiphe javanica*, *E. monoperidiata*, and *E. asiatica*.

CHARACTER	<i>E. javanica</i>	<i>E. monoperidiata</i>	<i>E. asiatica</i>
COLONIES	Hypophyllous	Amphigenous, mainly epiphyllous	Hypophyllous
HYPHAE (μm diameter)	3–6	4–6	4–6
CHASMOTHECIA (μm diameter)	(41–)50–66.5(–69.5) 2 asci	(55.5–)58–82.5(–85) 2–4 asci	(51–)57–74(–78) 2 asci
APPENDAGE (μm)	(8.5–)17.5–73.5(–95) $\times$ (2–)3.5–5.5(–8)	(15.5–)18–66(–75) $\times$ (2.5–)3–6(–7.5)	(31–)45–51(–66) $\times$ (4–)4.5–5(–5.5)
ASCI (μm)	(30.5–)36–50.5(–76) $\times$ (26–)27.5–44(–53) 5–7-spored	(34–)36–58(–61) $\times$ (24–)28–49(–52) 4–6-spored	(45–)46–59(–62) $\times$ (38–)40–53(–57.5) 6–8-spored
ASCOSPORES (μm)	(10.5–)11.5–24.1(–26) $\times$ (5–)8–14(–17)	(11–)12.5–25(–26) $\times$ (6–)7.5–13(–14.5)	(16–)18–25(–28) $\times$ (8.5–)9–15(–16.5)

Discussion

Among the seven *Erysiphe* species previously found on *Castanopsis* (Braun 1987; Divarangkoon et al. 2011), only *E. asiatica* and *E. monoperidiata* were reported to have a chasmothecial peridium composed of a single cell layer (Divarangkoon et al. 2011). Morphologically, there are only slight morphological differences between *E. javanica*, *E. asiatica*, and *E. monoperidiata* (TABLE 1). *Erysiphe javanica* differs from *E. monoperidiata* in having hypophyllous colonies, smaller chasmothecia (69 μm diam. in *E. monoperidiata*), having only 2 asci (with 5–7 spores per ascus) per chasmothecium, and being found only on *C. javanica* in Indonesia. In addition, the morphologically close *E. asiatica* differs slightly in having 6–8 spored asci and being confined to *C. diversifolia* and *C. echinocarpa* in Thailand. Although it is difficult to differentiate *E. javanica* from *E. asiatica* and *E. monoperidiata* solely on morphology, our phylogenetic analysis, using a combination of 28S and ITS rDNA sequences, clearly showed that *E. javanica* forms a distinct lineage distinguishable from *E. asiatica* and *E. monoperidiata* (FIG. 2).

Acknowledgments

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***Pisolithus*: a new species from southeast Asia
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ABSTRACT — Based on morphological and ITS nrDNA sequence analyses, a species recognized in classical European literature as *Lycoperdon capsulifer* is transferred to *Pisolithus* and *P. orientalis* is proposed as a new species. *Pisolithus orientalis* basidiomes, collected under *Pinus kesiya* in Thailand, correspond to *Pisolithus* sp. 5 sensu Martin et al. *Pisolithus* sp. 4 sensu Martin et al. is shown to comprise two groups, one equivalent to *P. arhizum* as collected from *Pinus* and *Quercus* forests from Italy and Spain and the other represented by the new *P. capsulifer* from mixed pine-deciduous forests in England and France.

KEY WORDS — ectomycorrhizal fungi, gasteromycete, phylogenetic species, PT, taxonomy

Introduction

Pisolithus is one of the most widespread and cosmopolitan gasteromycete genera ranging from temperate to tropical regions of the world. It has long been considered as monotypic (Coker & Couch 1928, Cunningham 1942, Pilát 1958) and is well-researched, primarily based on ectomycorrhizal studies, as *Pisolithus tinctorius* (P. Micheli ex Pers.) Coker & Couch (Marx et al. 1984, Burgess et al. 1995). Rauschert (1959) proposed that this species was conspecific with *Polysaccum arhizum* Scop., which he recombined as *Pisolithus arhizum* (Scop.) Rauschert. However, after molecular studies indicated that *P. tinctorius* is a species complex (Anderson et al. 1998, Cairney et al. 1999, Díez et al. 2001), Martin et al. (2002) proposed at least 11 phylogenetic species. Several subsequent morphological and molecular studies have confirmed

TABLE 1. *Pisolithus* specimens sequenced in the present study. * = Genbank sequences obtained from cultures.

TAXON	DNA CODE	HERBARIUM No.	ORIGIN	POTENTIAL HOST	COLLECTION DATE	GENBANK No.
<i>P. abditus</i> (sp. 12)	24PISOLI	BBH28595	Roi Et, Thailand	<i>Shorea roxburghii</i>	08-Jul-2001	FR748119
	31PISOLI	BBH28596	Yasothon, Thailand	<i>S. roxburghii</i>	13-Jun-2002	FR748120
<i>P. albus</i> (sp. 7)	12PISOLI	—	Yasothon Thailand	<i>Eucalyptus camaldulensis</i>	—	AF629887*
	13PISOLI	—	Petchaboon, Thailand	<i>E. camaldulensis</i>	29-Jan-2002	FR748121*
	14PISOLI	—	Chaiyapum, Thailand	<i>E. camaldulensis</i>	28-Jan-2002	FR748122*
	15PISOLI	—	Lumpoon, Thailand	<i>E. camaldulensis</i>	28-Jan-2002	FR748123*
	17PISOLI	—	Nakornsawan, Thailand	<i>E. camaldulensis</i>	28-Jan-2002	FR748124*
	20PISOLI	BBH28593	Waimea, Kona, HI, USA	<i>Eucalyptus</i> spp.	2002	FR748125
	21PISOLI	BBH28594	Australia	<i>Eucalyptus</i> spp.	2002	FR748126
	33PISOLI	BBH28599	Narathiwat, Thailand	<i>Melaleuca</i> spp.	2002	FR748127
<i>P. arhizus</i> (sp. 4)	3PISOLI	BCN-MPM2676	Tarragona, Spain	<i>Pinus</i> sp.	Oct-1997	FM213365
	92bisPISOLI	Wat.139161	Sardinia, Italy	—	Apr-2000	FR748128– FR748132
	6PISOLI	BCN-MPM2597	Tarragona, Spain	<i>Pinus</i> sp.	27-Nov-1996	FR748133
<i>P. capsulifer</i> (sp. 14)	51PISOLI	E00159834	Berkshire, England	—	Sep-1970	FR748134
	46PISOLI	E00290168 (epitype)	Berkshire, England	—	07-Sep-1993	FR748135
	49PISOLI	E00185017	Berkshire, England	<i>Pinus sylvestris</i> ; <i>P. contorta</i>	09-Sep-1973	FR748136
	53PISOLI	E00159822	Limoges, France	Mixed broadleaf & <i>Pinus</i> spp.	01-Sep-1993	FR748137
<i>P. tinctorius</i> (sp. 6)	5PISOLI	BCN-MPM2596	Alt Empordà, Girona, Spain	<i>Pinus</i> sp.	22-Jan-1995	FR748138
	19PISOLI	BBH28592	Juneau County, WI, USA.	<i>Quercus</i> & <i>Pinus</i> spp.	21-Sep-2001	FR748139
<i>Pisolithus</i> sp. 3	ARHPI3	MJ36192	Huelva, Spain	<i>Cistus</i> sp.	01-Jan-2003	FR748140– FR748142
<i>P. orientalis</i> (sp. 5)	7PISOLI	—	Chiang Mai, Thailand	<i>Pinus kesiya</i>	11-Aug-2001	FR748143*
	8PISOLI	—	Chiang Mai, Thailand	<i>P. kesiya</i>	11-Aug-2001	FR748144*
	9PISOLI	—	Petchaboon, Thailand	<i>P. kesiya</i>	28-Jan-2002	FR748145*
	10PISOLI	—	Petchaboon, Thailand	<i>P. kesiya</i>	28-Jan-2002	FR748146*
	11PISOLI	—	Petchaboon, Thailand	<i>P. kesiya</i>	28-Jan-2002	FR748147*
	33PISOLI	BBH28597 (holotype)	Chiang Mai, Thailand	<i>P. kesiya</i>	11-Aug-2001	FR748148
	34PISOLI	BBH28598	Chiang Mai, Thailand	<i>P. kesiya</i>	11-Aug-2001	FR748149

the taxonomic rank of some old species [*Pisolithus albus* (Cooke & Masee) Priest, *P. marmoratus* (Berk.) E. Fisch., *P. microcarpus* (Cooke & Masee) G. Cunn.] and established several new ones (*Pisolithus abditus* Kanch. et al., *P. aurantioscabrosus* Watling, *P. indicus* Natarajan & Senthil.). Nonetheless, some clades recognised by Martin et al. (2002) remain without specific names.

Additional morphological studies and ITS nrDNA sequence analyses of recently collected austro-asiatic, euro-asiatic, and paleotropical *Pisolithus* specimens have been conducted to assign morphological species to the *Pisolithus* sp. 5 phylogenetic lineage and to members of the AII clade of Martin et al. (2002).

Materials & methods

Fungal isolates

The *Pisolithus* material used in this work included fresh, herbarium, or cultured specimens sampled from a variety of geographical sites (TABLE 1). Basidiomes for each isolate are retained in the herbarium at the Microbiology Programme, Faculty of Science & Technology, Pibulsongkram Rajabhat University and BBH Thailand. Cultures obtained from basidiomata were grown and maintained on Modified Melin Norkrans' (MMN) medium (Marx & Kenny 1982) at 30°C with subculturing every 1–2 months. Herbarium specimens were provided by BCN and E (Abbreviations for herbaria follow Lanjouw & Stafleu 1964).

Molecular methods

Isolation, amplification, purification, and sequencing of DNA followed Phosri et al. (2007). DNA was isolated using the E.Z.N.A. fungi DNA miniprep kit (Omega Biotech, Doraville, Georgia, USA) as described by Martín & García-Figueres (1999) or a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). ITS regions were amplified with Ready-To-Go™ PCR Beads (Amersham-Pharmacia Biotech, NJ, USA) (Martín & Winka 2000). Primer pairs ITS1F/ITS4B and ITS1/ITS4 were used to amplify the ITS1+5.8S+ITS2 nrDNA gene cluster following White et al. (1990) and Gardes & Bruns (1993). Prior to sequencing, products were cleaned using either E.Z.N.A. Clean kit (Omega Biotech) or directly from the gel using QIAQuick PCR purification kit (QIAGEN, Chatsworth, CA). Both strands were sequenced separately at Secugen S.L. (Madrid, Spain) or Macrogen (South Korea). All samples were sequenced in both directions.

When needed, DNA was cloned using pGEM®-T Easy Vector System II cloning kit (Promega Corporation, Madison, Wisconsin, USA) and purified with QIAprep Spin Mini prep (QIAGEN). Both strands were sequenced separately using vector specific primers T7 and SP6 (Nag et al. 1988) at Secugen S.L. or Macrogen.

Sequence analysis

Navigator™ Sequence comparison software (Perkin Elmer Applied Biosystem) or Sequencher™ version 4.2 (Gene Codes Corporations, Ann Arbor, Michigan, USA) was used to assemble the consensus sequence from the two strands of the ITS nrDNA of each isolate. Blastn searches with megablast option were used to compare the sequences obtained against the sequences in the National Center of Biotechnology Information

(NCBI) nucleotide database. The new consensus sequences have been lodged in the EMLB-EBI database with accession numbers indicated in TABLE 1.

Sequences obtained were compared with homologous sequences of *Pisolithus* spp. retrieved from the EMBL Nucleotide Sequence Database with many published in Martin et al. (2002), Kanchanaprayudh et al. (2003a,b), Moyersoen et al. (2003), and Reddy et al. (2005) to represent all their described clades. Prior to 2009 sequences were aligned using the SEQAPP software (Perkin Elmer Applied Biosystem) and thereafter using Se-Al v2.0a11 Carbon (Rambaut 2002). The alignment was optimized visually. Alignment gaps were indicated as “-” and ambiguous nucleotides were marked as “N”.

Phylogenetic analyses

The alignment was analysed using the programme PAUP 4.0b10 (Swofford 2003) and MrBAYES v3.0 b4 (Huelsenbeck & Ronquist 2001) as described in Tellería et al. (2010). *Scleroderma citrinum* Pers. (FM213344) and *Suillus luteus* (L.) Roussel (GU373495) were included as outgroups. First, a maximum parsimony analysis (MP) was inferred using the heuristic search option in PAUP. Gaps were treated as missing data. Branch lengths equal to zero were collapsed to polytomies. Nonparametric bootstrap (BP) support (Felsenstein 1985) for each clade was tested based on 10000 replicates, using the fast-step option.

A second analysis was conducted using a Bayesian approach (Larget & Simon 1999, Huelsenbeck & Ronquist 2001). It was performed assuming the HKY + G model as suggested by hierarchical likelihood ratio test (hKRTs) and akaike information criterion (AIC) in MrModeltest 2.3 (Posada & Crandall 1998, Nylander 2004).

A combination of both posterior probabilities and bootstrap proportion was used to assess the level of confidence for a specific node (Lutzoni et al. 2004). The alignment matrix and the 50% majority-rule consensus tree from the Bayesian analysis are available in TreeBase (<http://www.treebase.org/>).

Recovered lineages and clades are named according to Martin et al. (2002). When a specific name has been formally assigned to a clade, the name is included along with the species number of Martin et al. (2002).

Results

Thirty-three new *Pisolithus* sequences have been generated, including those obtained from the epitype (E00290168) of the new combination, *P. capsulifer*, and the holotype (BBH28597) of the new species, *P. orientalis*. These were aligned with 67 additional sequences obtained from databases (GenBank and UNITE; see Figs 1–2) and phylogenetically analyzed.

The ITS nrDNA dataset contains 97 sequences and 893 aligned positions, of which 410 were constant, 177 parsimony uninformative, and 306 parsimony-informative. Maximum parsimony analysis yielded 100 most parsimonious trees (940 steps long, CI = 0.6681, HI = 0.3319, RI = 0.8968). One best tree found by branch swapping is shown in FIG. 1, with bootstrap values on the branches. The 50% majority-rule consensus tree from the Bayesian analyses is in FIG. 2, with posterior probabilities indicated on the branches.

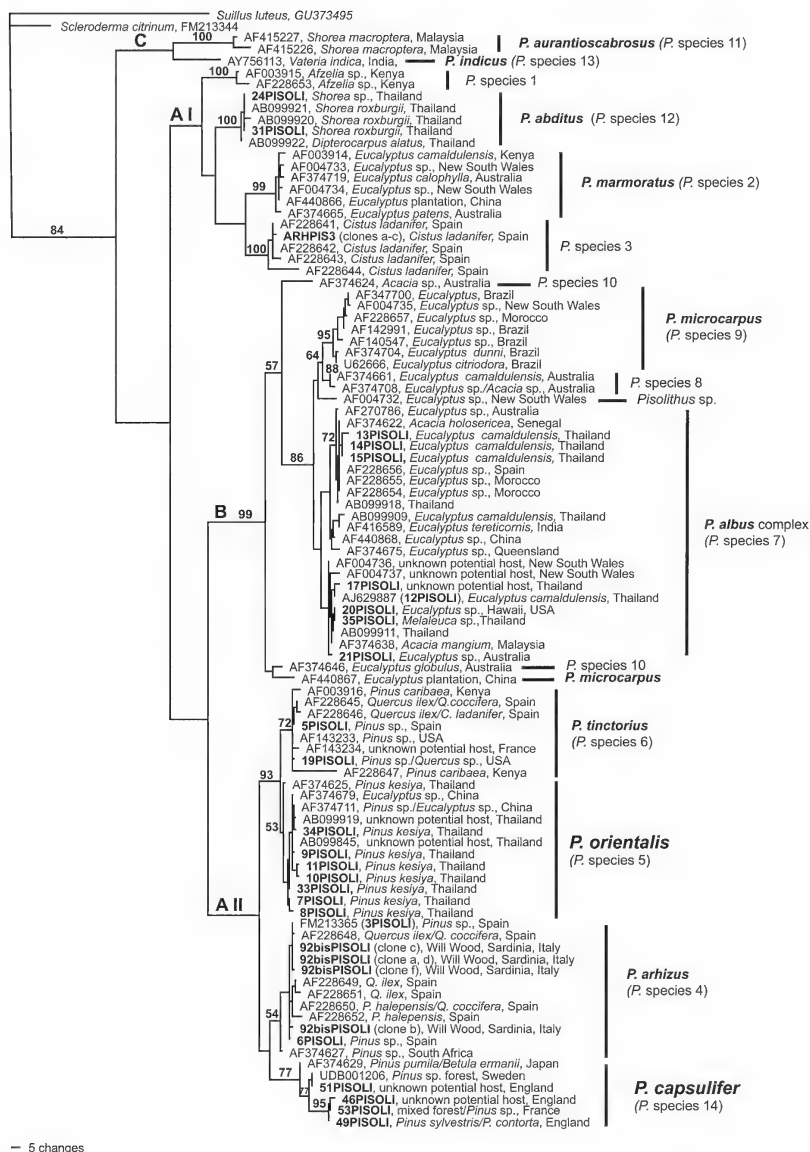


FIG. 1. Phylogenetic tree (one of 100 most parsimonious trees) obtained with a parsimony analysis under heuristic search of ITS sequences of *Pisolithus* collections included in TABLE 1 and sequences obtained from the GenBank and UNITE. Each branch is labeled with the DNA isolation code in bold (new sequences) or with the accession number including the possible host and the country. Numbers above branches represent bootstrap values. Clades and species number according to previous authors.

In both analyses, *Pisolithus* sequences form a highly supported clade (BS = 84%, PP = 1.0) with at least 15 terminal assemblages. However, the relationships between the clades are generally not well resolved and the main differences between FIG. 1 and FIG. 2 are related to the position of clades from lineage AI in Martin et al. (2002).

The basal clade (BS < 50%, PP = 0.62) (lineage C, Martin et al. 2002) included in our study contains *Pisolithus aurantioscabrosus* and *P. indicus*, which are sister to the remaining *Pisolithus* taxa. These two taxa are restricted to native dipterocarp forests in southeast Asia and India, respectively.

Lineage AI is separated into four clades, each with high support (BP $\geq$ 99% or PP = 1.0). Lineage AII also ramifies into at least four main groups, but these possess weak support (BS < 50%, PP = 0.98). The four AII groups are *Pisolithus* spp. 4, 5, and 6 of Martin et al. (2002), and one group here called *Pisolithus* sp. 14. *Pisolithus* sp. 6 (BS = 72%, PP = 0.99) consisted of eight *Pisolithus* sequences from basidiomata found mainly in association with pine (*Pinus caribaea* Morelet.) and oak (*Quercus ilex* L., *Q. coccifera* L.) in various regions ranging from USA, Spain, Kenya, and France. We consider this clade to be *P. tinctorius* s. st., sister to the *Pisolithus* sp. 5 clade formed by seven new isolates associated with *Pinus kesiya* Royle ex Gordon in Thailand and five GenBank sequences. The unifying features unique to these collections lead us to describe them below as a new species, *Pisolithus orientalis*. The other two clades (*Pisolithus* spp. 4 and 14) in Lineage AII are sister to the clade formed by *Pisolithus tinctorius* s. st. and *P. orientalis*. Collections from the British Isles (46PISOLI, 49PISOLI and 51PISOLI) and France (53PISOLI) clearly separate from *Pisolithus* sp. 4 (BS = 70%, PP = 1.0) and group together with sequences from Sweden (UDB001206) and Japan (AF374629). Sequenced specimens from British Isles, France, and Sweden have been analysed in parallel to basidiospore SEM studies. The electron micrographs (FIG. 3) indicate that the spore ornamentation does not resemble that of any other *Pisolithus* documented. The specific name *Pisolithus capsulifer* has been assigned to *Pisolithus* sp. 14.

Discussion

In lineage AII, one clade ('sp. 14') comprises predominantly British material. *Pisolithus* is very rare in the British Isles, where it is recorded from London, Hampshire, Devon, and Norfolk. Most records are from Tertiary gravels and sandy mineral soils and confined to the southernmost part, especially the southeast of England. There is one record of *Pisolithus* from Ireland 'on a bank in a car park, under *Castanea sativa* and *Pinus* spp., well-drained peat over lying sandy boulder-clay north slope of Knockmealdon Mountains, Kilballyboy Wd., South Tipperary (July 1984)' (Ing 1995), but it is not recorded from Scotland. The British sequences agreed with one each from France and Sweden. Here spore morphology is also distinctive, but is it significant? Grand (1976) and Watling

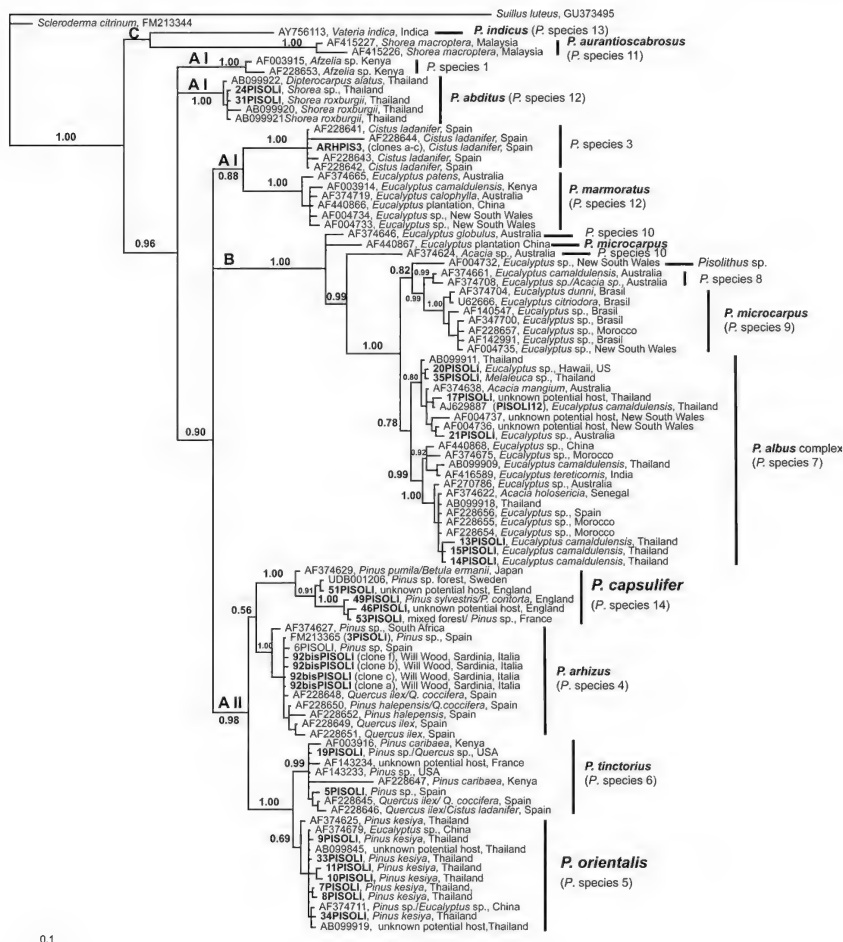


FIG. 2. Phylogenetic tree obtained with a Bayesian Monte Carlo Markov (MCMC) analysis assuming HKY + G model, as suggested by MrModeltest 2.3, of ITS nrDNA sequences of *Pisolithus* collections included in TABLE 1 and sequences obtained from the GenBank and UNITE. Each branch is labeled with the DNA isolation code in bold (new sequences) or with the accession number including the potential host and the country. Numbers above branches represent posterior probabilities values. Clades and species numbers according to previous authors.

et al. (1999) note that basidiospore morphology should be considered carefully as the mature spore ornamentation relies on spore development in which nurse cells play a vital role and affect final surface features. Morphological differences often have diagnostic importance for new morphological species, (e.g., *Astraeus odoratus*; Phosri et al. 2004, 2007), despite instances of convergence.

Sowerby (1814), who provided an excellent illustration of a gasteroid fungus, apparently coined the epithet '*Lycoperdon capsuliferum*' based on the fact that the fertile part of the fruiting body contained small spore capsules resembling a fig fruit. Fries (1829: 54) recognized the significance of this collection and how it differed from other species known to him mainly from the classical literature. He formally proposed the superfluous new name *Polysaccum olivaceum* in section *Radiculosa* for Sowerby's species, differing from the other members of the section in spore-mass colour and fruiting body shape. Because of the morphological and habitat similarities shared by Sowerby's fungus and modern 'sp. 14' clade collection, we are confident in identifying this material as *Lycoperdon capsuliferum*, which we transfer to *Pisolithus*.

***Pisolithus capsulifer* (Sowerby) Watling, Phosri & M.P. Martín, comb. nov.** FIG. 3

MYCOBANK MB 519949

[*PISOLITHUS* SP. 14]

= *Lycoperdon capsuliferum* Sowerby, Col. Fig. Engl.

Fung., Suppl.(no. 31): pl. 425a/b. 1814.

= *Polysaccum olivaceum* Fr., Syst. Mycol. 3(1): 54. 1829 [nom. nov. superfl.].

TYPE: Coloured plate 425a/b in Sowerby, Col. Fig. Engl. Fung., Suppl.(no. 31), 1814 (lectotype designated here). ENGLAND, Berkshire, Sandhurst, 7 Sep 1993, leg. E.E. Green, Wat. 25310 (epitype designated here, E00290168; GenBank FR748135)

BASIDIOMES turbinate to pyriform with subglobose to ellipsoid head 40–60 mm diam. or flattened at the apex > 90 mm broad, tapering into solid, firm, irregular pseudostipe incorporating soil debris; pseudostipe 15–30(–90) mm long often flattened and then 10–25 mm broad truncated downwards to 15 mm. PERIDIUM wafer thin, > 1mm thick, dry, smooth, yellowish ochraceous with olivaceous spots and faint netted appearance from outline of pseudoperidioles below, then fragmenting to expose powdery spore-mass and disintegrating pseudoperidioles. Lower part more compacted and with an olivaceous-yellow hue, finally drying hard after weathering to become clinker-like. PSEUDOPERIDIOLES pale then red-brown finally burnt umber, larger at apex and compressed downwards, becoming pulverulent.

CLAMP-CONNECTIONS present. BASIDIOSPORES 6.8–8.2–10.6 µm, globose, spinose spines at first separate and 1.1–1.9–2.9 µm long, before collapsing to coalesce into groups and falling back onto spore surface to give a rivulose effect.

ADDITIONAL SPECIMENS EXAMINED: FRANCE, 20 km. from Limoges, mixed broadleaf-conifer forest, 1-Sep-1997, leg. E.E. Green, Wat. 25298 (E00159822). UNITED KINGDOM, HAMPSHIRE, Ringwood, 12-Sep-1926, coll. E.J.H. Corner 81444 (E00075037); coll. E.J.H. Corner 81446 (E00075038); BERKSHIRE, Windsor Great Park, Jul-1993, leg. E.E. Green, Wat. 25311 (E00290167); Caesar's Camp near Bracknell, Sep-1970, leg. E.E. Green (E00159834); 9-Sep-1973, leg. E.E. Green, Wat. 10118 (E00185017); 3-Oct-1981, leg. E.E. Green, Wat. 14216 (E00185018).

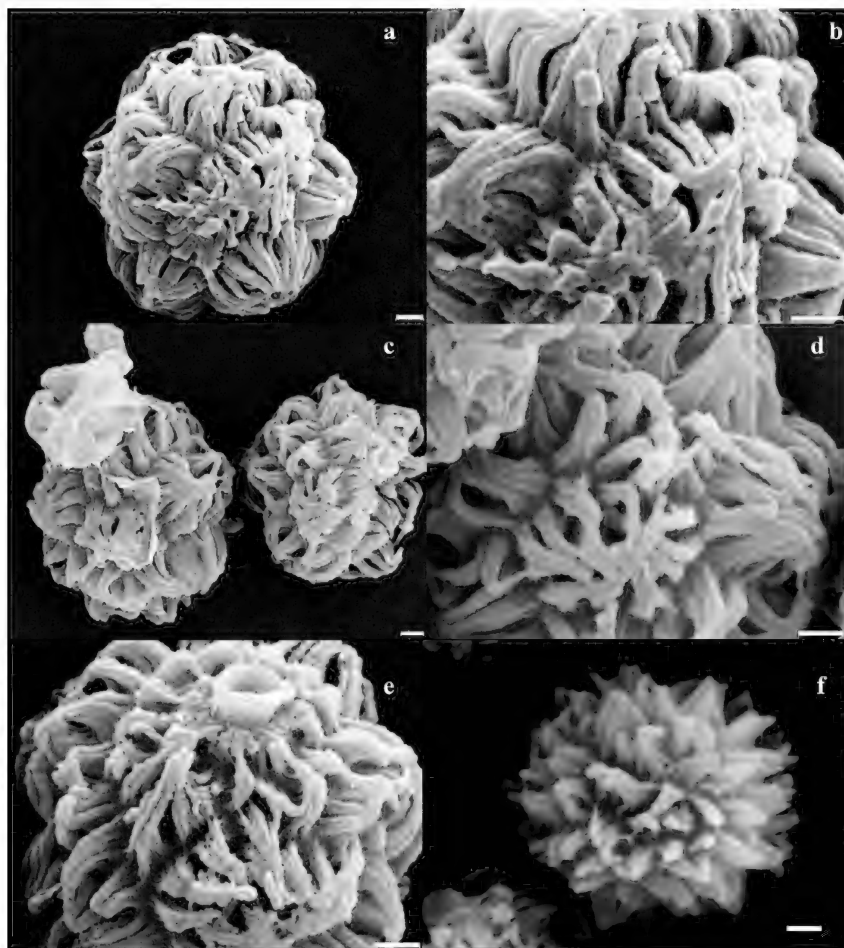


FIG. 3. SEM of spore ornamentation of selected *Pisolithus* collections (bars = 1 μ m). a–e: *P. capsulifer*. From England—a–b: epitype E00290168 (Wat. 25310, 46PISOLI), c–d: E00185017 (Wat. 10118, 49PISOLI); from France—e: E00159822 (Wat. 25298, 53PISOLI). f: *P. orientalis*. Holotype BBH28597 (33PISOLI).

COMMENTS. The distribution of the pseudoperidioles in fresh material is clearly expressed by Sowerby (1814) when he wrote, “at first strong and very rugged but the congeries of seeds, if I may so-call them, were enveloped or compressed into forms laying by each other, giving a reticulated appearance in some directions, being mostly rather oblong, and of a dark brown colour...and looks like pollen bursting from something analogous to anthers.”

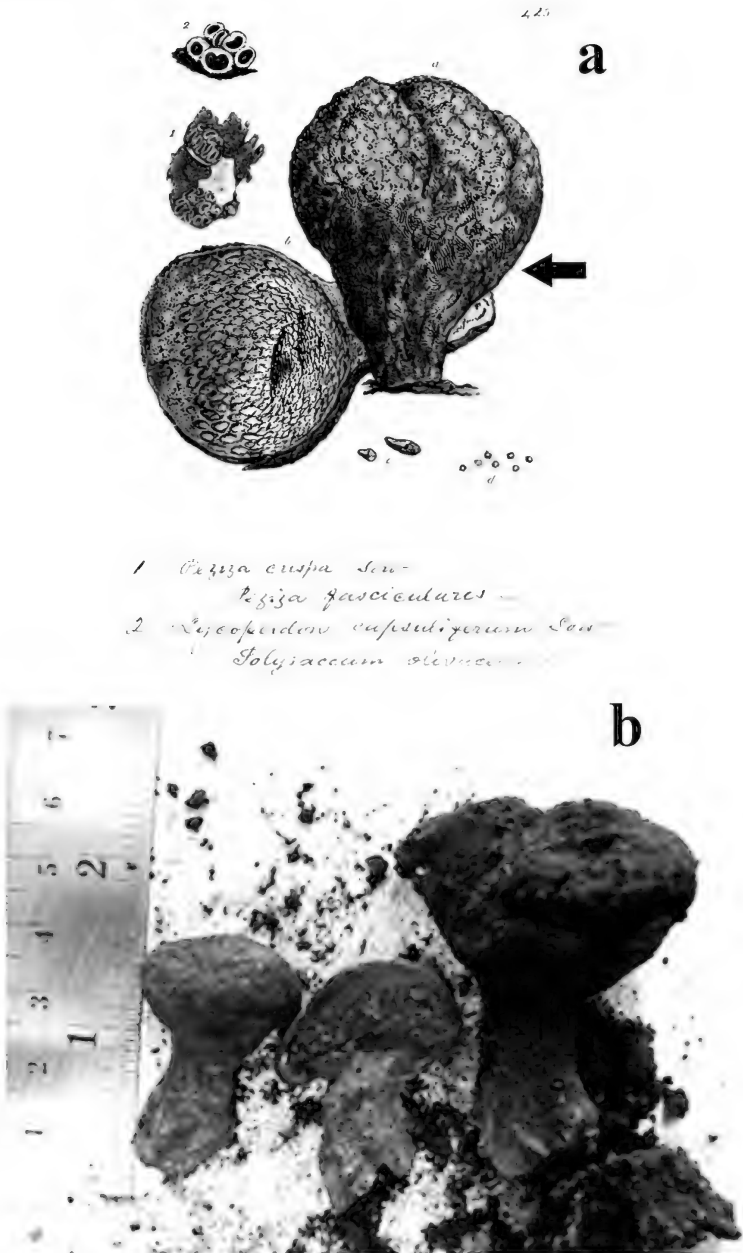


FIG. 4. a: Sowerby's illustration of *Lycoperdon capsuliferum* (arrow).
b: *Pisolithus orientalis* Holotype BBH28597 (33PISOL1).

***Pisolithus orientalis* Watling, Phosri & M.P. Martín sp. nov.**

FIG. 4

MYCOBANK MB519952

[*PISOLITHUS* SP. 5]

Differs from *Pisolithus tinctorius* by smooth pedicellate basidiomata with blackish brown discs, smaller globose basidiospores ornamented by isolated groups of narrow cones that adhere together to form secondary spines, and an association with *Pinus kesiya*.

TYPE: THAILAND. Chang Mai, Hot District, Bo Kaew pine plantation, under *Pinus kesiya*, 11-Aug-2001 (holotype, BBH 28597; GenBank FR748148).

BASIDIOME: 35–55 mm high, pedicellate to broadly clavate, divided into distinct head and cylindrical stipe, snuff-brown to cigar-brown with a black shining surface around the head, surface smooth, not scaly. PERIDIUM: 24–45 mm broad, broadly ellipsoid to subglobose, rough, thin, brittle, snuff-brown, breaking up at the apex to expose powdery contents and join up to stipe. STIPE: 13–24 × 10–15 mm woody, snuff-brown, smooth, cylindrical base of agglutinate mycelium and soil, yellowish rhizomorphs when immature. GLEBA: snuff-brown to ferruginous powdery mass formed by the breakdown of peridioles seated in a small black pocket within the peridium. PERIDIOLES: thin-walled, ovoid-ellipsoid, smooth, clay-buff, 2 × 1 mm, at first clay-buff then more snuff-brown or ferruginous as they mature, discrete, but later breaking down into a snuff brown to ferruginous powdery mass, exuding sienna to fulvous fluid when mounted in alkaline solution.

CONSTITUENT HYPHAE: intertwined, hyaline to ochraceous, thin-walled, 2.4–4.8 mm broad, septate without encrustation, clamp-connections present. BASIDIA not seen. BASIDIOSPORES: globose, 5.8–9.0–10.7 µm including spines, heavy ornamentation consisting of isolated groups of connate, narrow cones adhering together to form points, lacking connections.

ADDITIONAL SPECIMENS EXAMINED: THAILAND, Chiangmai, 11-Aug-2001 (BBH28598; GenBank FR748149), together with other specimens referred to *P. orientalis* (sp. 5) in

TABLE 1. Other sequences obtained were directly from cultures.

The pedicellate, smooth basidiomes that become blackish brown at the disc above the first maturing pseudoperidioles, the relatively small spores that are covered with narrow cones that join together to form connate secondary spines, and an association with *Pinus kesiya* distinguish *Pisolithus orientalis* from other species in the genus. The distinctive spore ornamentation coupled with the molecular data distinguishes this new taxon. The characters emphasis how important it is to examine in detail the development of both basidiome and basidiospores in *Pisolithus*.

Conclusion

Our study confirms that *Pisolithus tinctorius* sensu lato, as previously and widely understood, covers several separate species. Molecular work linked to scanning electron microscope studies of the basidiospores is proving

essential in unravelling speciation within *Pisolithus*. The marriage of data from classical sources and our molecular and morphological studies confirms the distinctiveness of the British, French, and Swedish collections and a new species from SE Asia growing with *Pinus kesiya*.

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***Terriera simplex*, a new species of *Rhytismatales* from China**XIAO-MING GAO¹ CHUN-TAO ZHENG¹ & YING-REN LIN^{2*}¹ School of Life Science & ² School of Forestry & Landscape Architecture,

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ABSTRACT —A new *Terriera* species found on fallen leaves of *Trachelospermum jasminoides* (Apocynaceae) is described, illustrated, and designated as *Terriera simplex*. This taxon is distinguished from its closest relatives by unbranched paraphyses and asci with truncate or subtruncate apices. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

KEY WORDS —taxonomy, Ascomycota, morphology, vine plant

Introduction

The genus *Terriera* B. Erikss. was established by Eriksson (1970) for *T. cladophila* (Lév.) B. Erikss., belonging to *Rhytismataceae* (Kirk et al. 2008). Minter (1996) provided a detailed description of the type species.

Over the years, however, some species which should belong to *Terriera* have been mistakenly placed in *Clithris* (Fr.) Bonord., *Dermascia* Tehon, *Hypoderma* De Not., and (particularly) *Lophodermium* Chevall. (Johnston 2001). Johnston (2001), who divided *Lophodermium* on monocotyledonous plants into groups A, B, C, and D, regarded Group B species as closer to the type species of *Terriera* based on their special ascomatal structure. He therefore transferred 11 species and 1 variety of *Lophodermium* Group B to *Terriera* as new combinations and published three new species. The author also invalidly published a fourth species, in that work, which he later validly published as *T. samuelsii* P.R. Johnst. (Johnston 2003). In the paper by Ortiz-García et al. (2003), Johnston recombined *Clithris minor* Tehon as *T. minor* (Tehon) P.R. Johnst. rDNA-ITS sequence analyses by Ortiz-García et al. (2003) indicated that *Lophodermium* from pine hosts were distally related to *Terriera* and that two monophyletic *Terriera* sequences formed a weakly supported sister group related to *Lirula macrospora* (R. Hartig) Darter.

Five *Terriera* species have previously been reported from China: *T. brevis* (Berk.) P.R. Johnst., *T. huangshanensis* Z.Z. Yang et al., *T. camelliae* (Teng) Y.R. Lin & Jiang L. Chen, *T. coacervata* Y.R. Lin & Q. Zheng, and *T. illiciicola* (S.J. Wang et al.) Q. Zheng & Y.R. Lin (Fröhlich & Hyde 2000, Yang et al. 2011, Chen et al. 2012, Zheng et al. 2012). In the present paper, we describe a new species of *Terriera* on confederate jasmine, a vine from the Dabie Mountains in Anhui Province, China.

Materials & methods

External characteristics of conidiomata and ascomata obtained from open ascomata of the holotype specimen were observed under a dissecting microscope at 10–50×. After rehydration in water for 10 min, 10–15 µm thick sections of the conidiomata and ascomata were cut using a freezing microtome. Microscopic examinations were made in water, 5% KOH, Melzer's reagent, or 0.1% (w/v) cotton blue in lactic acid. For observing the outlines of ascomata and conidiomata in vertical section, sections were mounted in lactic acid or cotton blue with pretreatment in water. Gelatinous sheaths surrounding ascospores and paraphyses were observed in water or cotton blue in lactic acid. The color of internal structures and ascospore contents were observed in water. Measurements and drawings of asci, ascospores, and paraphyses (30 per specimen) were made using material mounted in 5% KOH or Melzer's reagent. Point and line integrated illustrations of external shapes and internal structures of the conidiomata and ascomata were prepared using a microscope drawing device.

Taxonomy

Terriera simplex Y.R. Lin, X.M. Gao & C.T. Zheng, sp. nov.

FIGS 1–6

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Differs from *T. cladophila* in unbranched paraphyses and asci with truncate or subtruncate apices and from *T. minor* by the presence of conidiomata and ascomata with obtuse, rounded or slightly acute ends.

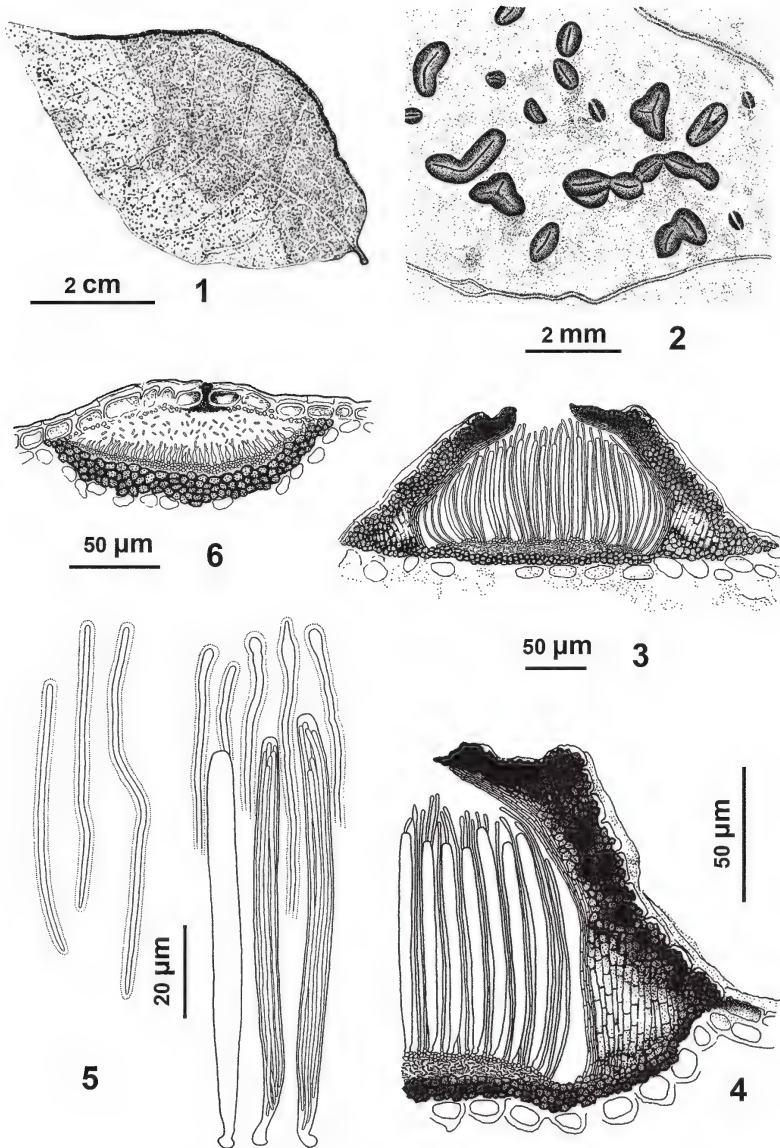
TYPE: on fallen leaves of *Trachelospermum jasminoides* (Lindl.) Lem. (*Apocynaceae*), China, Anhui, Dabie Mountains, Tiantangzhai, alt. ca 700 m, 16 September 2005, S.J. Wang and Y.R. Lin 2020 (Holotype AAUF 68128).

ETYMOLOGY: *simplex*, referring to the simple paraphyses, which are not branched near the apex.

ZONE LINES absent.

CONIDIOMATA on both sides of leaves, crowded or sometimes coalescent.

In surface view, conidiomata 130–200 µm diam., rounded or subrounded, black-brown in the centre, more or less concolorous with the substratum surface elsewhere, slightly raising the leaf surface, discharging spores through a 3–5 µm diam. apical ostiole. In vertical section, conidiomata subepidermal, lenticular. UPPER WALL very poorly developed, composed of tiny, thin-walled globose cells 1.6–2 µm diam. BASAL WALL well developed, 12–17 µm thick, consisting of 2–4 (–5) layers of angular cells 2–4.5 µm diam. SUBCONIDIogenous LAYER 4.5–8



FIGS 1–6. *Terriera simplex* on *Trachelospermum jasminoides*. 1. A leaf bearing fruit bodies. 2. Conidiomata and ascomata observed under a dissecting microscope. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci and ascospores. 6. Conidioma in vertical section.

μm thick, composed of textura angularis with nearly colorless, thin-walled cells. CONIDIOGENOUS CELLS $6\text{--}11 \times 2\text{--}3 \mu\text{m}$, subcylindrical, tapering to the apex, proliferating sympodially. CONIDIA $2.5\text{--}4 \times \text{ca } 1 \mu\text{m}$, cylindrical or nearly elliptical, hyaline, unicellular.

ASCOMATA in similar positions to conidiomata on the substratum, crowded in irregular, light yellow to grayish-white, large bleached areas without obvious edges, sometimes 2–6 ascomata coalescent. In surface view, ascomata $650\text{--}1000 \times 350\text{--}480 \mu\text{m}$, elliptical to ovate, ends obtuse, rounded or slightly acute, black, slightly shiny, with a clearly marked outline, moderately raising the surface of the leaf, opening by a single longitudinal split which is sometimes branched in the triangular ascomata. Immature ascomata seen as two parallel black patches on the substratum surface rather than as a single more or less elliptical one, with a pale central zone along future line of opening. Lips absent; split extending $3/4\text{--}4/5$ the length of the ascoma. In median vertical section, ascomata subepidermal, with epidermal cells becoming filled with fungal tissue as ascomata develop, $120\text{--}135 \mu\text{m}$ deep. COVERING STROMA $18\text{--}22 \mu\text{m}$ thick near the opening, black-brown, composed of textura angularis with thick-walled cells $3\text{--}5.5 \mu\text{m}$ diam. Along the edge of the ascoma opening, there is a flattened, $8\text{--}12 \mu\text{m}$ thick extension adjacent to the covering stroma, and which comprises strongly carbonized tissue with no obvious cellular structure. EXCIPULUM very poorly developed, arising from the inner layer of the basal stroma, consisting of hyaline textura porrecta. BASAL STROMA $6\text{--}15 \mu\text{m}$ thick, dark-brown, composed of 2–3(–4) layers of $3\text{--}5 \mu\text{m}$ diam., angular, thick-walled cells. $20\text{--}32 \mu\text{m}$ thick textura prismatica with somewhat thin-walled, nearly colorless to grayish-brown cells exists between the covering stroma and basal stroma. SUBHYMENIUM moderately developed, $7\text{--}12 \mu\text{m}$ thick, consisting of hyaline textura angularis and intricata. PARAPHYSES $95\text{--}110 \times 1.2\text{--}1.5 \mu\text{m}$, filiform, aseptate, not branched, occasionally gradually or abruptly swollen to $2.5\text{--}3 \mu\text{m}$ near the apex where sometimes agglutinated, covered in a $\text{ca } 1 \mu\text{m}$ thick gelatinous matrix. ASCI ripening synchronously, $72\text{--}95(105) \times 4.8\text{--}5.2 \mu\text{m}$, cylindrical, very short-stalked, thin-walled, apex truncate, subtruncate or obtuse, without circumapical thickening, J–, 8-spored. ASCOSPORES arranged in a fascicle, $(45\text{--})56\text{--}82 \times 1\text{--}1.2 \mu\text{m}$, filiform, slightly tapered towards the rounded base, hyaline, with a gelatinous sheath $0.8\text{--}1 \mu\text{m}$ thick.

HOST SPECIES & DISTRIBUTION: producing conidiomata and ascomata on fallen leaves of *Trachelospermum jasminoides*; known only from the type locality, Anhui, China.

COMMENTS—We place our new species in *Terriera* based on the presence of a strongly carbonized extension adjacent to the ascoma opening and textura prismatica in the corner between the covering and basal stroma and the absence of lip cells.

Terriera simplex is distinguished from the type species *T. cladophila* by its sometimes coalescent and occasionally three-lobed ascomata, paraphyses with unbranched upper parts, and asci with truncate or subtruncate apices. While ascomata in *T. cladophila* are scattered and sometimes circular, the paraphyses are frequently and irregularly branched and irregularly twisted or swollen at the apex, and the tips of asci are rather rounded (Minter 1996). *Terriera minor*, a widely distributed species, resembles *T. simplex* but differs in ascomata with rounded ends that are not associated with conidiomata, paraphyses that branch 2–3 times in the upper 30–40 µm, and sequentially maturing asci (Johnston 1988, 1989a,b).

Ascomata of *T. simplex* are mature on dead leaves. It is not known whether this fungus occurs in living leaves as a mutualistic endobiont or as a parasite. This needs to be investigated.

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The *Leptogium juressianum* complex in southeastern BrazilMARCOS J. KITaura^{1*} & MARCELO P. MARCELLI²¹UNESP, Instituto de Biociências, Depto de Botânica,

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ABSTRACT— *Leptogium subjuressianum* and *L. subjuressianum* var. *caparoense* are new Brazilian taxa growing mostly in the southeastern mountains. They are differentiated from European taxa by the ornamentation of the upper surface.

KEY WORDS— lobules, margin, granular isidia

Introduction

Leptogium juressianum is the only species in *Leptogium* sect. *Mallotium* producing isidia-like structures and with hairs confined mostly to the upper surface (Jørgensen 1997, Tavares 1950). The other *Leptogium* species with hairs chiefly on the upper surface — *L. eriodermoides* Arv. & P.M. Jørg. and *L. resupinans* Nyl. (Jørgensen 1997) — do not have ornamented thalli.

Such hairs, initially observed by Tavares (1950), are commonly considered an important taxonomic characteristic and have a specific organization. However, the organization is rarely described in detail and sometimes not even mentioned (Swinscow & Krog 1988, Galloway & Jørgensen 1995).

Jørgensen & James (1983) described the hairs of *L. juressianum* as an arachnoid tomentum while Jørgensen (1997) called them cobwebby hairs. Tavares (1950) described the holotype from Portugal as producing isidiate or lacinulate propagules, but later specimens with alternative propagules were also identified as *L. juressianum*: cylindrical to complanate isidia and lacinulae were noted for Ethiopian (Africa) collections (Swinscow & Krog 1988), western European specimens produced granular to sublobulate isidia (Jørgensen & James 1983), and the upper surface of specimens from Chile were found to have granular, sublobulate, or lacinulate isidia and isidiate-lacerate lobe margins (Galloway & Jørgensen 1995).

Leptogium specimens collected during the first author's doctoral studies, were initially identified as *L. juressianum*. Upon type revision and under our descriptive protocol we discovered that the material represents new taxa, which are described here, together with comments on *L. juressianum*.

Material & methods

This study is mainly based on material collected in the Serra da Mantiqueira, a major mountain complex in southeastern Brazil, as well as on the isotype of *L. juressianum* (UPS). We were unable to study the holotype because the Museu Nacional de História Natural (LISU) in Lisboa, Portugal, did not respond to a loan request.

Comparative morphological and anatomical characteristics summarized in TABLE 1 are modeled after a table first assembled by Cunha (2007), who studied the *Leptogium* species collected in forests (mainly mangroves) and urban littoral habitats of São Paulo state. The character set has been modified to encompass the morphological variations we observed in the Serra da Mantiqueira taxa.

The nature of the thalline surfaces and their ornamentation are considered important taxonomic characters. The surfaces are described without and with magnification; the system of folds and/or wrinkles and their size is determined by complex organization of the hyphae (both cortical and internal), which remain constant in both dry and wet specimens. Some folds are readily seen with the naked eye, but smaller folds (≤ 0.1 mm, which appear smooth to the naked eye but can readily be seen under magnification $\geq 10\times$) must be observed under the stereomicroscope.

Several species apparently bear similar propagules on the upper surface, but careful observation has shown that their morphology and position are good species characters. Thus descriptions of their form, dimensions, distribution, and ontogeny are described in TABLE 1.

TABLE 1. Morphological comparison of *Leptogium juressianum*, *L. subjuressianum*, and *L. subjuressianum* var. *caparoense*.

TAXON	ORNAMENTATION	ORNAMENT DISTRIBUTION	HAIR DISTRIBUTION	DRY THALLUS COLOR*
<i>L. juressianum</i>	Cylindrical when young to flattened when fully developed	Usually on lobe margins	Upper surface; parts of underside	Gray to bluish where glabrous
<i>L. subjuressianum</i> var. <i>subjuressianum</i>	Granular isidia and lobules	Usually on lobe margins	Upper surface and usually on underside margin	Brownish where glabrous
<i>L. subjuressianum</i> var. <i>caparoense</i>	Granular isidia	Abundant on lamina; frequent on lobe margins	Frequent on upper surface; abundant on underside	Dark brown to blackish where glabrous

* All whitish where hairy

All specimens analyzed were dissected under a stereomicroscope with a razor blade and the semi-permanent slides were mounted with solution of 50% glycerine.

Results & discussion

The name *Leptogium juressianum* has been attributed to all ornamented (bearing isidia-like or lobule-like structures) specimens of *Leptogium* that develop hairs mostly on the upper surface (Tavares 1950, Jørgensen & James 1983, Jørgensen & Nash 2004). The detailed descriptions of thallus and ornamentation produced according to our protocols have enabled us to distinguish the Brazilian taxa. This was not possible using brief descriptions and the characters commonly used to describe and differentiate *Leptogium* species. Thallus color, hair distribution, and the nature of the vegetative propagules are the principal diagnostic characters in this paper (TABLE 1).

Leptogium juressianum Tav., Port. Acta Biol., Ser. B, 3: 68, 1950.

FIGS 1–2

TYPE: PORTUGAL, Minho [corresponding with the modern districts of Braga and Viana do Castelo], Serra do Gêres, between Pedra Bela and the Caldas, 700 m alt., on *Arbutus unedo*, 16.VI.1947, leg. C. Tavares 2081 (holotype, LISU; isotype, UPS!).

THALLUS gray, opaque, matt, gray to whitish when observed under the stereomicroscope. LOBES irregularly lacerate, to 3 mm wide, overlapping, attached in points, convolute, upper surface smooth and velvety under the naked eye, pubescent at 10× and higher magnifications (interlaced hairs that are similar to sponge fibers); apices rounded, usually ascending to involute, smooth or isidiate to lacinulate; lateral margin fimbriate (isidiate to lacinulate), ascending, irregular; lower side bluish gray, smooth at different magnifications. ISIDIA absent. LACINULES smooth, cylindrical when young to flattened, ca. 0.08–0.30 × 0.03–0.08 mm, unbranched to irregularly branched but not coralloid, erect, firm, concolorous with the thallus, usually marginal, dense, frequent to abundant, grouped on the lamina. ATTACHED by hairs; rhizines absent; hapters absent; hairs irregular, unbranched or branched, up to 125 µm (ca. 9 cells) long, dense on upper surface, frequent on lower side. APOTHECIA absent.

ANATOMY— THALLUS ca. 60 µm thick, quadratic cells of the cortices ca. 7.5 × 5.0 µm; columnar hyphae ca. 2.5 µm thick, straight to inclined, 2(–3) cells. CYANOBACTERIA blue, frequent to abundant, cell number not appraised, spherical, 5 µm diam.; gelatinous matrix poor, hyaline. PYCNIDIA absent.

COMMENTS— *Leptogium juressianum* is characterized by the isidiate to lacinulate margins and hairs on the upper surface.

Originally described from Portugal, the species has been cited for western Europe (Jørgensen & James 1983), southern Africa (Swinscow & Krog 1988), and Chile, South America (Galloway & Jørgensen 1995), but always with different type and/or position of propagules. Since the propagules are major

diagnostic characters, we believe the previously cited collections may represent different taxa and that all that material needs re-evaluation

Leptogium subjuressianum Marcelli & Kitaura, sp. nov.

FIGS 3–4

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Differs from *Leptogium juressianum* by granular isidia and presence of rounded lobules.

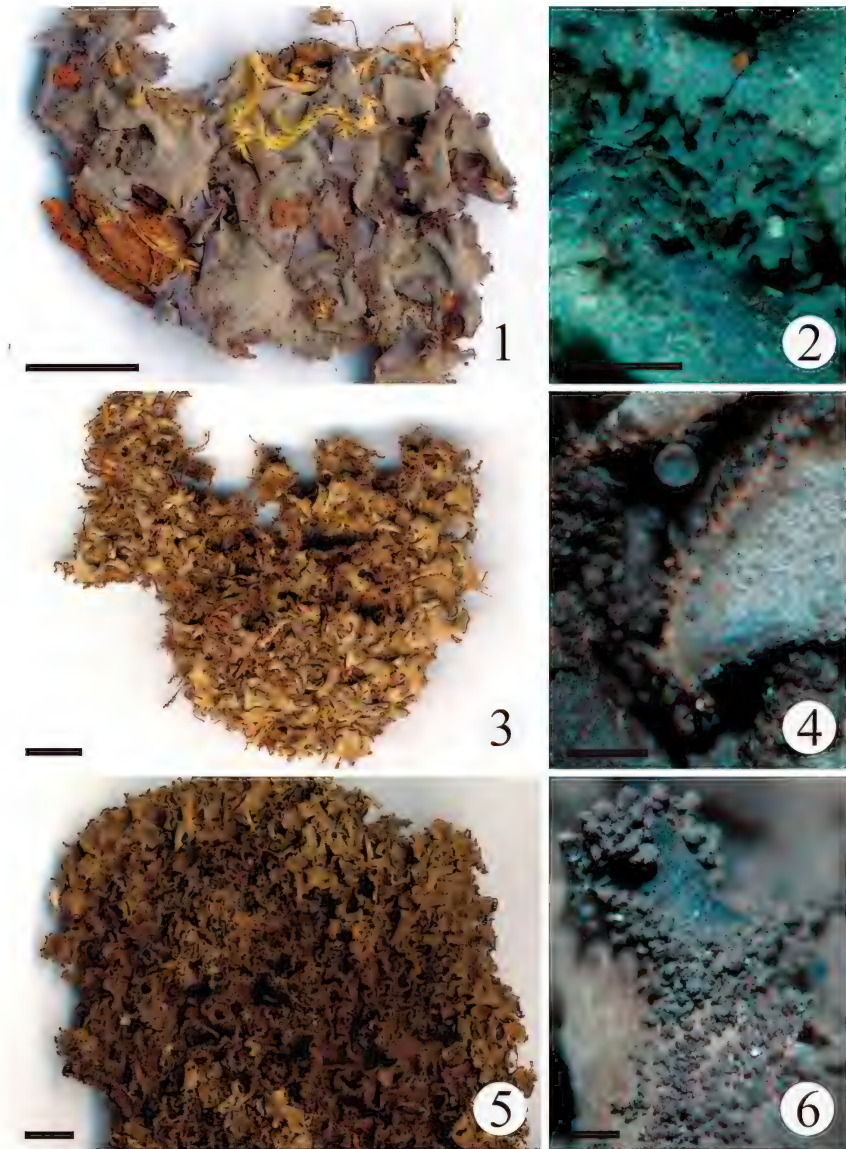
TYPE: Brazil, Rio Grande do Sul State, municipality of Tapes, on trunk of a tree, 5 m alt, 29.I.1994, leg. M.P. Marcelli 26459 (holotype, SP).

ETYMOLOGY: The specific epithet refers to the closely related species, *L. juressianum*, the name first applied to the studied material.

THALLUS whitish or black (naked eye), opaque, matt, whitish brown when observed under the stereomicroscope. LOBES to 2 mm wide, irregularly overlapping, attached in points, ascending; upper surface velvety when observed with the naked eye, pubescent at 10× and higher magnifications; apices rounded, ascending, involute to convolute, ornamented with small granular to lobulate isidia; lateral margins lobulate, ascending or flat, undulate; lower side bluish gray, smooth, covered by long hairs seen at 10× and higher magnification. ISIDIA granular, ca. 0.05 mm diam., unbranched, erect, firm, concolorous with the thallus, rare on the lamina but dense at the margins. LOBULES with apices rounded, ca. 0.5 mm diam., unbranched, erect, firm, concolorous with the thallus, marginal, dense, abundant. ATTACHED by rhizines and hairs; hapters absent; rhizines unbranched, beige, evenly distributed, frequent, formed from agglutinated hairs; hairs interlaced, unbranched or branched, ≤ 125 µm (ca. 8 cylindrical cells) long, beige, present on both surfaces, abundant on the upper surface and frequent on the lower surface. APOTHECIA absent.

ANATOMY— THALLUS ca. 55 µm thick, upper quadratic cells ca. 10 × 10 µm, lower quadratic cells ca. 5 × 5 µm, columnar hyphae ca. 2.5 µm thick, straight and inclined, comprising 3–4 cells. CYANOBACTERIA blue, frequent, cell number not appraised, cells spherical, ca. 5 µm diam.; gelatin frequent, colorless. PYCNIDIA absent.

ADDITIONAL MATERIAL EXAMINED: **BRAZIL. PARANÁ STATE**, General Carneiro Municipality, Fazenda Lageado Grande, s/d, S. Eliasaro 2892 (UPCB); **SÃO PAULO STATE**, Campos do Jordão Municipality, Parque Estadual de Campos do Jordão, *Araucaria/Podocarpus* forest, on tree trunk inside the forest, 1400 m alt., 17.VI.1995, M.P. Marcelli, A. Gugliotta & R. Maziero 28914 (SP); **MINAS GERAIS STATE**, Camanduia Municipality, on trunk, 21.XI.2008, M.J. Kitaura, M.P. Marcelli, A.E. Luchi & S.N. Inoue 1158 (SP), 1179 (SP); 22.XI.2008, M.J. Kitaura, M.P. Marcelli, A.E. Luchi & S.N. Inoue 1232 (SP), 1235 (H); Itamonte Municipality, BR-485, on trunk, 22.I.2009, M.J. Kitaura & M.P. Marcelli 1468 (SP); Brejo da Lapa, on trunk, 23.I.2009, M.J. Kitaura & M.P. Marcelli 1536 (SP); **ESPÍRITO SANTO STATE**, Dorcas do Rio Preto Municipality, on trunk, 16.IX.2009, M.J. Kitaura & M.P. Marcelli 1792 (SP), 1804 (SP), 1825 (BOTU); on branch fallen on the rock, 16.IX.2009, M.J. Kitaura & M.P. Marcelli 1911 (SP).



FIGURES 1–6. *Leptogium juressianum* (isotype, UPS): 1. Thallus; 2. Propagules. *L. subjuressianum* var. *subjuressianum* (holotype): 3. Thallus; 4. Propagules. *L. subjuressianum* var. *caparoense* (holotype): 5. Thallus; 6. Propagules. Scale bars: 1, 3, 5 = 5 mm; 2, 4, 6 = 20 μ m.

COMMENTS— *Leptogium subjuressianum* is characterized by the rounded lobules on the thallus margin and spongioid hairs on the upper surface, which usually appears whitish due to the presence of dense hairs. Both *L. juressianum* and *L. subjuressianum* are covered by a dense layer of spongioid hairs, which are interlaced and resemble fibers of sponges.

According to Tavares (1950), *L. juressianum* possesses only isidia and lacinules on the margins. No rounded lobules were observed in any part of the isotype. We did not find lacinules in any specimens of *L. subjuressianum*, where only granular to lobulate isidia are present prior to the development of lobules.

Leptogium subjuressianum is common in Brazil and is usually collected on tree trunks. Specimens were collected from Tapes Municipality, Rio Grande do Sul State (30°40'12"S 51°23'24"W) to Alto Caparaó Municipality in Minas Gerais State (20°29'42"S 41°59'54"W).

***Leptogium subjuressianum* var. *caparoense* Kitaura & Marcelli, var. nov.**

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FIGS 5–6

Differs from *Leptogium subjuressianum* var. *subjuressianum* by the isidia distribution and the presence of rhizomorphic hyphae on the lower surface.

TYPE: Brazil, Minas Gerais State, Municipality of Alto Caparaó, Cachoeira Bonita, on organic material on rock beside the waterfall, 17.IX.2009, leg. M.J. Kitaura & M.P. Marcelli 1900 (holotype, SP; isotypes, H).

ETYMOLOGY: The varietal epithet refers to the Caparaó Range, a major mountain complex in southeastern Brazil where the types were collected.

THALLUS whitish to blackish (naked eye), opaque, matt, whitish (hairy part) and dark brown or black (glabrous part) when observed under the stereomicroscope. LOBES to 3 mm wide, irregular, overlapping, attached in points, ascending, upper surface smooth or velvety when observed with the naked eye, pubescent, smooth or ornamented at 10× and higher magnifications; apices rounded, ascending or involute, usually smooth; lateral margins smooth to granular, ascending, undulate; lower side usually brownish, with adhering substrata when observed with the naked eye, smooth or less pubescent than upper surface at 10× and higher magnifications. ISIDIA granular, ca. 0.1 mm diam., concolorous with the thallus, firm, dense or abundant on the lamina and frequent at the margins. LOBULES rounded, ca. 0.5 mm diam., unbranched, erect, firm, concolorous with the thallus, marginal when the thallus is covered by hairs, frequent or rare on the lamina. ATTACHED by rhizines and hairs; hapters absent; rhizines unbranched, beige, evenly distributed, frequent, constituted from agglutinated hairs; hairs interlaced, irregular, beige, dense on upper surface and frequent on lower surface, cells cylindrical. APOTHECIA absent.

ANATOMY— THALLUS ≤ 70 µm thick, upper quadratic cells (wall slightly blackish) ca. 10 × 10 µm, lower quadratic cells ca. 7.5 × 7.5 µm; columnar hyphae

ca. 2.5 µm thick, straight to inclined, 4-celled. CYANOBACTERIA yellow to slight blue, frequent, cells spherical, ca. 5 µm diam.; gelatin frequent to scarce, yellow next to upper cortex and colorless below. PYCNIDIA absent.

ADDITIONAL MATERIAL EXAMINED: BRAZIL, MINAS GERAIS STATE, Alto Caparaó Municipality, Cachoeira Bonita, saxicolous, 17.IX.2009, M.J. Kitaura & M.P. Marcelli 1902 (H), 1905 (SP); on the moss on the rock, 17.IX.2009, M.J. Kitaura & M.P. Marcelli 1904 (SP), 1932 (BOTU).

COMMENTS— *Leptogium subjuressianum* var. *caparoense* is characterized by granular isidia on both the lamina and margins (older parts) and the blackish or dark brown colored thallus (when not covered by spongoid hairs).

The lobules common on the margins of *L. subjuressianum* var. *subjuressianum* were not observed in *L. subjuressianum* var. *caparoense* (TABLE 1).

Furthermore, the isidia in *L. subjuressianum* var. *caparoense* are mainly laminal but primarily marginal in *L. subjuressianum* var. *subjuressianum*. Also, *L. subjuressianum* var. *caparoense* has rhizomorphic hyphae covering the entire lower surface whereas in var. *subjuressianum* they are present mainly at the margins of the lobes.

All specimens of *L. subjuressianum* var. *caparoense* were collected in an especially humid place on wet rocks beside a waterfall inside Caparaó National Park. We attribute to this the abundance of rhizomorphic hyphae and spreading of the propagules and hairs and prefer to retain the taxon at the varietal level.

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***Corioloopsis psila* comb. nov. (*Agaricomycetes*) and two new *Corioloopsis* records for Brazil**

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ABSTRACT — The new combination *Corioloopsis psila* is proposed and *C. brunneoleuca* and *C. hostmannii* are reported as new to Brazil. Descriptions of these collections and a key to the eight accepted *Corioloopsis* species reported from Brazil are provided.

KEY WORDS — Polyporaceae, diversity, *C. byrsina*, *C. caperata*, *C. floccosa*

Introduction

Corioloopsis Murrill (Murrill 1905) comprises about 17 valid species (Kirk et al. 2008). The genus is cosmopolitan, although several species are restricted to the temperate or tropical zones (Ryvarden & Johansen 1980, Ryvarden & Gilbertson 1993, Núñez & Ryvarden 2001, Dai 2011, Dai et al. 2011). It is characterized by the annual (rarely perennial) pileate or sessile (rarely resupinate) basidiomata with great color variation. The hyphal system is trimitic with clamp connections on generative hyphae, cystidia are absent, and basidiospores are cylindrical, smooth, thin-walled, non-amyloid, oblong–ellipsoid, and hyaline to pale brown (Ryvarden & Johansen 1980, Ryvarden 1991, Ryvarden & Gilbertson 1993).

Only seven *Corioloopsis* species have previously been reported from Brazil: *C. aspera* (Jungh.) Teng, *C. badia* (Berk.) Murrill, *C. burchellii* (Berk. ex Cooke) Ryvarden, *C. byrsina*, *C. caperata*, *C. floccosa*, and *C. gallica* (Fr.) Ryvarden (Ryvarden 1988, Gugliotta & Abrahão 2011).

To increase the knowledge of polypore diversity in Brazil, we record two additional species, propose a new combination, and provide identification keys to all known Brazilian species.

Material & methods

In the Brazilian Amazonia, field trips were undertaken in Pará State from July 2006 to February 2008 in the Floresta Nacional de Caxiuanã (1°42'3"S 51°31'45"W) and in Rondônia State from 2007 to 2008 in the Estação Ecológica de Cuniã (8°04'S 63°31'W) and Parque Natural Municipal de Porto Velho (8°45'S 63°54'W). In Pernambuco State, Brazilian mangroves were surveyed from March 2009 to March 2010 in Maria Farinha (7°51'24.8"S 34°50'32.7"W), Itamaracá (7°46'52.6"S 34°52'53.3"W), Maracaípe (8°32'22.8"S 35°00'29.1"W), and Rio Formoso (8°41'20.8"S 35°06'06.6"W).

Both recent collections and specimens deposited in INPA, O, RB, and SP were studied. Specimens were analyzed macro- (shape, color, hymenial surface) and micromorphologically (hyphal system, presence/absence and measurements of sterile structures and basidiospores). Slides were prepared with 5% KOH (stained with 1% aqueous phloxine) or Melzer's reagent (Ryvarden 1991). Color designation followed Watling (1969). The material was incorporated to HFSL, MG, O, and URM.

Results & discussion

After identification of new collections and revision of herbaria, eight *Corioloopsis* species are reported for Brazil. The presence of *C. gallica* is not confirmed, as previous reports actually represent *Hexagonia hydroides* (Sw.) M. Fidalgo (Tavares 1939, URM 608/IPA 281) or *Trametes* sp. (Bononi et al. 1984, SP 156751). *Corioloopsis badia* is a dubious species because it is considered a synonym of either *Phellinus badius* (Cooke) G. Cunn. (<http://www.cbs.knaw.nl>, <http://www.mycobank.org>) or *C. aspera* (Ryvarden & Johansen, 1980). The only record of *C. badia* in Brazil (Gibertoni et al. 2004, URM 77849) represents a *Trametes* sp.

Corioloopsis brunneoleuca and *C. hostmannii* are new occurrences for the country. *Corioloopsis psila*, here transferred from *Fomes*, is probably a new occurrence for the Brazilian Amazonia. *Corioloopsis byrsina* represents a new record for the states of Amazonas, Rondônia, and Roraima, *C. caperata* for Amazonas, and *C. floccosa* for Acre, Amazonas, and Mato Grosso. These new occurrences were studied also from collections deposited in INPA, RB and SP, underscoring the importance of herbarium revisions and accessibility of herbaria records to the knowledge of fungal diversity.

Corioloopsis brunneoleuca (Berk.) Ryvarden, Norw. Jl Bot. 19: 230 (1972).

BASIDIOMATA annual, effused-reflexed, gregarious, coriaceous, ≤ 5.5 cm, 1.5 cm wide and to 1 mm thick. ABHYMENIAL SURFACE tomentose, dull, cigar brown (16) to fuscous black (36) with concentric zones of dark color. MARGIN entire, acute, concolorous with the abhymenial surface. Context homogeneous, fibrous, ≤ 100 µm thick, milky coffee (28) to cinnamon (10). HYMENIAL SURFACE with pores angular, 2–3 per mm, dissepiments 50–100 µm thick, snuff brown (17) to clay buff (32). HYPHAL SYSTEM trimitic; generative hyphae hyaline, branched, clamped, thin-walled, 1.5–2.5 µm diam.; skeletal hyphae yellowish to

pale brown, thick-walled, 4–7.5 µm diam., dextrinoid; binding hyphae golden-yellow, almost solid, 3–5 µm diam. CYSTIDIA absent. Basidia not observed. BASIDIOSPORES cylindrical, hyaline, thin-walled, smooth, inamyloid, 7–11 × 3–4 µm.

ECOLOGY & DISTRIBUTION: On deciduous wood. According to Ryvarden & Johansen (1980), the species is pantropical. It is new to Brazil.

SPECIMENS EXAMINED: **BRAZIL. PARÁ**, Floresta Nacional de Caxiuanã, VIII.2007, leg. P.S. Medeiros et al. PS122, PS226, PS342 (MG195152, MG195155, MG195182).

REMARKS: A cigar brown to fuscous black pileus surface, rather large shallow pores, and broad dextrinoid skeletal hyphae characterize the species.

Corioloopsis byrsina (Mont.) Ryvarden, Norw. Jl Bot. 19: 230 (1972).

DESCRIPTION: This species is characterized by small pores, soft rusty-brown basidiomata, and ellipsoid to sub-cylindrical basidiospores, 9–14 × 4.5–5 µm. For further details see Ryvarden & Johansen (1980).

ECOLOGY & DISTRIBUTION: On deciduous wood. In tropical Africa, America, and Asia (Ryvarden & Johansen 1980; Dai 2011). In Brazil, previously known from the states of Acre, Mato Grosso, Rio Grande do Sul, and São Paulo (Gugliotta & Abrahão 2011); newly reported from Amazonas, Rondônia, and Roraima.

SPECIMENS EXAMINED: **BRAZIL. Acre**: Rio Branco, 24.IX.1980, leg. B. Lowy et al. 241 (INPA 183806); **AMAZONAS**: Presidente Figueiredo, 18.I.1983, leg. M.A. Jesus 123 (INPA 183806); **MATO GROSSO**: Aripuanã, 23.IV.1978, leg. M.A. Sousa 427 (INPA 75606); **RONDÔNIA**: Jarú, Reserva Biológica de Jarú, 13.V.1987, leg. M. Capelari et al. (SP 211995); location not determined, 4.VII.1968, leg. K.P. Dumont et al. 143 (INPA 65179); 3.II.1984, leg. G.J. Samuels 55 (INPA 129095); **RORAIMA**: locality unknown, 1.XII.1977, leg. I.J. Araújo et al. 757 (INPA 78476); **SÃO PAULO**: Itaic, 06.VII.1957, leg. not determined (URM 7880, as *Polystictus byrsinus*).

Corioloopsis caperata (Berk.) Murrill, N. Amer. Fl. 9(2): 77 (1908).

DESCRIPTION: *Corioloopsis caperata* is characterized by effused-reflexed to pileate darkly colored basidiomata and a zonate abhymenial surface in different shades of brown. For further details see Ryvarden & Johansen (1980).

ECOLOGY & DISTRIBUTION: On deciduous wood. Tropical Africa and America (Ryvarden & Johansen 1980). In Brazil, it is reported for the states of Acre, Alagoas, Amapá, Bahia, Espírito Santo, Mato Grosso, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Rio Grande do Norte, Rondônia, Roraima, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo, Sergipe (Baltazar & Gibertoni 2009, Gugliotta & Abrahão 2011), and now for Amazonas State.

SPECIMENS EXAMINED: **BRAZIL. ACRE**: Sena Madureira, 27.IX.1968, leg. G. T. Prance et al. 7595 (INPA 24394); **AMAZONAS**: Barcelos, II.1984, leg. G. J. Samuels 72 (INPA 129121); Borba, 6.V.1985, leg. K.F. Rodrigues et al. 490 (INPA 129022, as *Coriolus versicolor*); Manaus, 1.V.1977, leg. M. A. Souza 149 (INPA 74657, as *Corioloopsis polyzona*); 6.X.1985, leg. K.F. Rodrigues et al. 800 (INPA 137086); 5.X.1927, leg. P. Occhioni (SP 25530); 23.XII.1983, leg. G. Guzman et al. (SP 193577); Manicoré, IV.1985, leg. K.F. Rodrigues et al. 91 (INPA 128914, as *Corioloopsis* sp); Nova Aripuanã, 29.IV.1985, leg.

K.F. Rodrigues et al. 410, 389 (INPA 129009, as *C. polyzona*, INPA 129005, as *Polyporus* sp.); Tapuruquara, 22.I.1978, leg. I. J. Araújo et al. 973 (INPA 78745, as *Hexagonia* sp.); Tefé, 11.VII.1973, leg. E. Lleras et al. 16611 (INPA 39800); Cachoeira de Tarumã, 5.X.1927, leg. P. Occhioni (RB 217241); Serra de Araçá, 29.II.1984, leg. G. J. Samuels (RB 24785); Manicoré, Estrada do Estanho, 20.IV.1985, leg. K.F. Rodrigues (RB 238166); Novo Aripuanã, Vila do Apuy, 29.IV.1985, leg. K.F. Rodrigues et al. (RB 238334); Distrito Agropecuário da Suframa, 03.X.1985, leg. N. Rodrigues et al. (RB 237012, RB 240587); **PARÁ**: Belém, 13.IX.1968, leg. B. Santiago (SP 106707); 3.VI.1980, leg. V. L. Bononi (SP 177467); Melgaço, VIII.2007, leg. T. B. Gibertoni (URM 79656 on *Xylopia* sp.; URM 79653 on *Pouteria* sp.; URM 79650 on *Nectandra* sp.; URM 79649 on *Licania* sp.; URM 79646 on *Parkia* sp.; URM 79647 on *Sclerolobium* sp.; URM 79648 on *Eschweilera* sp.; URM 79652 on *Dinizia excelsa*); Redenção, 20.VIII.1984, leg. N. Rodrigues (RB 224462); Oriximiná, 28.VI.1980, leg. V.L. R. Bononi et al. 638, 241 (INPA 103609, as *Coriolus* sp., INPA 103335, as *Trametes pinsita*); Itaituba, IX.1977, leg. M.A. Sousa et al. 25 (INPA 74631, as *Hexagonia caperata*); **PERNAMBUCO**: Escada, 26.X.1954, leg. not determined (URM 1010, as *Polystictus occidentalis*); Recife, 24.VII.1957, leg. not determined (URM 12468, as *P. caperatus*); Tapera, 1932, leg. not determined (URM 762, as *P. caperatus*); **RONDÔNIA**: Caracará, 3.VII.1968, leg. G.T. Prance et al. 5497 (INPA 22257); Campo Novo, X.1979, leg. R.H. Petersen 155 (INPA 110517, as *Polyporus* sp.); Jarú, 2.X.1986, leg. M. Capelari & R. Maziero (SP 211273); Vilhena, X.1979, leg. R. H. Petersen 218 (INPA 110706, as *Polyporus* sp.); Porto Velho, Estação Ecológica de Cuniã, II.2007, leg. A. C. Gomes-Silva 33 (URM 78898); II.2008, leg. A. C. Gomes-Silva 222 (URM 78906); 11.III.2010, leg. A.C. Gomes-Silva et al. 815 (URM 82837); Parque Natural Municipal de Porto Velho, VII.2007, leg. A. C. Gomes-Silva 59 (URM 78900); VII.2008, leg. A.C. Gomes-Silva 603 (URM 79552); 20.VI.2009, leg. A.C. Gomes-Silva et al. 738 (URM 82833); 29.I.2010, leg. A.C. Gomes-Silva et al. 941 (URM 82831); 3.II.2011, leg. A.C. Gomes-Silva et al. 1089 (URM 82903); Fazenda Mucum, VII.2007, leg. A.C. Gomes-Silva 91 (URM 78901); **RORAIMA**: Alto Alegre, 21.VI.1986, leg. K.F. Rodrigues et al. 1083 (INPA 143425); Caracará, 16.XI.1977, leg. I. J. Araújo et al. 438 (INPA 76933, as *Hexagona* sp.).

Corioloopsis floccosa (Jungh.) Ryvarden, Norw. Jl Bot. 19(3–4): 230 (1972).

DESCRIPTION: The umber brown tomentose to hirsute or even velutine pileus surface and fairly large pores are diagnostic for this species. For further details see Ryvarden & Johansen (1980).

ECOLOGY & DISTRIBUTION: On deciduous wood. Pantropical (Ryvarden & Johansen 1980). In Brazil, reported for the states of Alagoas, Bahia, Pará, Paraíba, Paraná, Pernambuco, Rio Grande do Sul, Rondônia, Roraima, Santa Catarina and São Paulo (Gugliotta & Abrahão 2011). Newly recorded for Acre, Amazonas, and Mato Grosso.

SPECIMENS EXAMINED: **BRAZIL:** **ACRE:** location not determined, 3.X.1980, leg. B. Lowy et al. 1069 (INPA 100886, as *Polyporus* sp.); **AMAZONAS:** Manaus, 10.X.1989, leg. R. Carvalho 1332 (INPA 192503, as *Corioloopsis* sp.); 21.VI.1985, leg. M. A. Jesus 755 (INPA 185984); 10.IX.1977, leg. M. A. Sousa & I. J. Araújo 219 (INPA 74661, as *Coriolus maximus*); Itacoatiara, 21.XII.1966, leg. G. T. Prance et al. 3778 (INPA 19403, as *Corioloopsis* sp.); 10.IX.1980, leg. B. Lowy et al. 129 (INPA 100040, as *Polyporus* sp.); **BAHIA:** Conde, 08.II.1955, leg. A.C. Batista (URM 1242, as *Polystictus pinsitus*); Amaro, 23.V.1957, leg. not determined (URM 7450, as *Polystictus membranaceus*); **MATO**

GROSSO: Aripuanã, 23.IV.1978, leg. M.A. Sousa et al. 431 (INPA 75610); **PARÁ:** Belém, 17.IV.1979, leg. I.J. Araújo 1239 (INPA 102851); Itaituba, 3.X.1977, leg. M.A. Sousa et al. 140 (INPA 74538, as *Corioloropsis* sp.); **PERNAMBUCO:** Recife, 23.VII.1955, leg. not determined (URM 12498, as *P. membranaceus*); **RIO GRANDE DO SUL:** São Leopoldo, 1932, leg. J. Rick (URM 9023, as *Polystictus rigens*); **RORAIMA:** Alto Alegre, 16.VI.1986, leg. E.S.S. Silva et al. 493 (INPA 154958); Boa Vista, 19.VII.1989, leg. M.A. Jesus 880 (INPA 186185, as *Corioloropsis* sp.); 1.XII.1977, leg. L.L.J. Aguiar et al. 750 (INPA 78469, as *Corioloropsis* sp.); 31.I.1984, leg. G.J. Samuels 39 (INPA 129089); **RONDÔNIA:** Porto Velho, Estação Ecológica de Cuniã, VII.2007, leg. A.C. Gomes-Silva 204 (URM 78909); Parque Natural Municipal de Porto Velho, VII.2007, leg. A.C. Gomes-Silva 186 (URM 79477, as *Corioloropsis rigida*); Fazenda Mucum, VII.2007, leg. A.C. Gomes-Silva 258 (URM 79478, as *C. rigida*).

Corioloropsis hostmannii (Berk.) Ryvarden, Syn. Fung. 23: 39 (2007).

BASIDIOMATA perennial, sessile, hard and brittle when dry, dimidiate to flabelliform, solitary, projecting 2–4 cm, 3–5 cm wide and 0.5–1.5 mm thick. **ABHYMENIAL SURFACE** glabrous, dull to subshiny, zonate with brown and purple zones, hazel (27) to drab (33). **MARGIN** acute, concolorous with abhymenial surface. **CONTEXT** homogeneous, ≤ 0.5 mm thick, fulvous (12) to cigar brown (16). Tubes concolorous with context, ≤ 0.5 mm long. **HYMENIAL SURFACE** with pores round, 6–7 per mm, dissepiments 50–100 μm thick, cigar brown (16). **HYPHAL SYSTEM** trimitic, generative hyphae hyaline, clamped, thin-walled, 1.5–2.5 μm diam.; skeletal hyphae yellowish to pale brown, thick-walled, 4–7.5 μm diam.; binding hyphae golden-yellow, almost solid, 3–5 μm diam. **CYSTIDIA** absent. **BASIDIA** not observed. **BASIDIOSPORES** cylindrical, hyaline, thin-walled, inamyloid, $8\text{--}10 \times 2.5\text{--}4.0$ μm .

ECOLOGY & DISTRIBUTION: On deciduous wood. Neotropical, from Southern United States and south to Venezuela (Ryvarden & Johansen 1980). It is new to Brazil.

SPECIMENS EXAMINED: **BRAZIL. PERNAMBUCO:** Paulista, Manguezal de Maria Farinha, on dead *Rhizophora mangle*, 20.VIII.2009, leg. G.S. Nogueira-Melo et al. NM003 (URM 82147); on dead *Avicennia schaueriana*, 31.I.2010, leg. G.S. Nogueira-Melo et al. NM002 (URM 82146); Itamaracá, Manguezal do Canal de Santa Cruz, on live *A. schaueriana*, 25.VI.2009, leg. G. S. Nogueira-Melo et al. NM002, NM025 (URM 82134, URM 82138); on dead *A. schaueriana*, 10.III.2009, leg. G.S. Nogueira-Melo et al. NM001, NM004 (URM 82136, 82145); 26.VI.2009, leg. G.S. Nogueira-Melo et al. NM003, NM007, NM011 (URM 82144, URM 82143, 82137); 26.VII.2009, leg. G.S. Nogueira-Melo et al. NM002 (URM 82139); 22.VIII.2009, leg. G.S. Nogueira-Melo et al. NM006, NM012 (URM 82141, URM 82140); 17.I.2010, leg. G.S. Nogueira-Melo et al. NM002 (URM 82135); 19.III.2010, leg. G.S. Nogueira-Melo et al. NM004 (URM 82142).

REMARKS: *Corioloropsis hostmannii* may be confused with *C. aspera*, which has similar hyphae and basidiospores. However, *C. hostmannii* has smaller pores (3–4 pores per mm in *C. aspera*) and smooth pilei. The zonate abhymenial surface may cause the species to be mistaken for *Funalia polyzona* (Pers.) Niemelä, although *Funalia* usually has a distinct variably zoned tomentum.

***Corioloopsis psila* (Lloyd) Ryvarden, comb nov.**

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= *Fomes psila* Lloyd, Syn. Fomes: 233 (1915). [BPI!].

= *Phylloporia psila* (Lloyd) Ryvarden, Norw. JI Bot. 19: 235 (1972).

BASIDIOMATA annual to perennial, sessile, solitary, sessile, pileus dimidiate to flabelliform, ≤ 6 cm in diam. and 2 cm thick with the tomentum, the latter as a dense mat of intertwined hairs in reddish brown colours, ≤ 1 cm thick. CONTEXT cigar brown (16) to umber (18), ≤ 4 mm thick in lower denser part, no black zone towards the cottony dense hair surface mat, tubes single layer or stratified as in the type, individual layer 3–4 mm thick. HYMENIAL SURFACE rusty-brown; dissepiments entire, relatively thick; pores round, 6–7 per mm, almost invisible to the naked eye. HYPHAL SYSTEM trimitic, generative hyphae often collapsed and difficult to observe, hyaline, thin-walled, but always with a distinct lumen often with secondary adventitious septa, $\leq 8 \mu\text{m}$ in diam., binding hyphae irregular in outline, frequently branched or with a few long tapering branches, thick-walled, yellow to light brown, $\leq 6 \mu\text{m}$ in diam. BASIDIOSPORES cylindrical, $9\text{--}12 \times 3\text{--}4.5 \mu\text{m}$, hyaline, thin-walled and non-amyloid.

ECOLOGY & DISTRIBUTION: On deciduous wood. Known from Brazil and Mexico. The type locality in Brazil is not detailed but J. Rick, who collected the material, used to work in Rio Grande do Sul State, South Brazil. If so, it is probably a new occurrence for the Brazilian Amazonia and Northeast Brazil.

SPECIMENS EXAMINED: **BRAZIL. AMAZONAS:** Manaus, 20.IV.1985, leg. M.A. Jesus 709 (INPA 185947, as *Perenniporia* sp.); 2.V.1990, leg. M.A. Jesus 1384 ([INPA 192652, as *Microporus* sp.); Manicoré, 20.IV.1985, leg. K.F. Rodrigues 233 (INPA 128955, as *Polyporus* sp.); **BAHIA:** location not determined, date not determined, leg. C. Torrend (O 10486); Gongogi, V.1910, leg. C. Torrend, s.n. (URM 8893, as *Trametes ocellata* Berk. & M.A. Curtis); **RONDÔNIA:** location not determined, 21.IX.1980, leg. B. Lowy et al. 230 (INPA 185947, as *Perenniporia* sp.); 20.V.1987, leg. M. Capelari et al. (SP 212021).

EXTRALIMITAL SPECIMEN EXAMINED: **MEXICO. OAXACA:** Tehuantepec, 13.VI.1976, leg. A.L. Welden 3606 (O 10488).

REMARKS: *Corioloopsis psila* is a conspicuous species due the dense thick mat of entangled reddish brown hairs covering the pileus. Superficially it reminds one of *H. hydroides*, which, however, has black and stiffer hairs on the pileus. Basidiospores of the two species are similarly cylindrical, but those of *H. hydroides* are longer ($12\text{--}14 \mu\text{m}$). The generic concepts among polypores with a trimitic hyphal system producing white rot (*Corioloopsis*, *Hexagonia*, *Pycnoporus*, *Trametes*) are unclear and are based in part on basidioma color, a character of dubious value at the generic level. Further DNA sequencing will reveal the true phylogeny within these genera. For the time being we place *F. psila* in *Corioloopsis* due to its brown context and a spore length that is closer to other *Corioloopsis* species than in *Hexagonia*.

The combination in *Phylloporia* was based on the presence of a black line between the hairy cover of the pileus and context, a prominent character for

Phylloporia species. However *Phylloporia* was later characterized by small ellipsoid basidiospores and a parasitic life strategy (Wagner & Ryvarden 2002), while *C. psila* is saprophytic and has larger basidiospores. Seemingly a biological convergent character, the hairy pilear covering occurs in many polypore genera and is apparently an adaption to resist drying.

Key to the species of *Corioloropsis* recorded in Brazil

- 1a. Basidiomata mostly sessile 2
- 1b. Basidiomata mostly effused-reflexed 5
- 2a. Pores 1–4 per mm 3
- 2b. Pores 6–7 per mm 4
- 3a. Abhymenial surface finely tomentose at the margin, pores angular,
1–2 per mm, basidiospores not known *C. burchellii*
- 3b. Abhymenial surface with scrupose tuft of agglutinated hairs, pores round,
3–4 per mm, basidiospores cylindrical $9\text{--}16 \times 3\text{--}5 \mu\text{m}$ *C. aspera*
- 4a. Abhymenial surface glabrous, basidiospores $8\text{--}10 \times 2.5\text{--}4 \mu\text{m}$ *C. hostmannii*
- 4b. Abhymenial surface with a dense mat of intertwined hairs,
basidiospores $9\text{--}12 \times 3\text{--}4.5 \mu\text{m}$ *C. psila*
- 5a. Basidiospores oblong ellipsoid to subcylindrical, $10\text{--}14 \times 4.5\text{--}6 \mu\text{m}$,
hyaline to very pale yellowish *C. byrsina*
- 5b. Basidiospores ellipsoid to cylindrical, fusiform, hyaline, $7\text{--}11\text{--}(14) \times 2\text{--}4 \mu\text{m}$ 6
- 6a. Context distinctly duplex, tobacco-brown and shiny. *C. floccosa*
- 6b. Context mostly homogeneous, dark brown to bay and dull 7
- 7a. Context distinctly darker than the tubes, hymenial surface bay to deep brown,
pores round to angular, (2–)4–5 per mm *C. brunneoleuca*
- 7b. Context dark date-brown concolorous with tubes, hymenial surface ochraceous,
to cinnamon to deep chocolate brown, pores round, 3–5 per mm *C. caperata*

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A new species of *Postia* (Basidiomycota) from Northeast China

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ABSTRACT — A new polypore, *Postia subplacenta* sp. nov. from Northeast China, is described and illustrated. It is characterized by resupinate basidiocarps, angular pores (3–5 per mm) with a cream buff to pale cinnamon-buff pore surface, and cylindrical basidiospores ($4.2\text{--}6.0 \times 1.9\text{--}2.4 \mu\text{m}$) that are negative in Melzer's reagent and Cotton Blue. A key to accepted species of Chinese *Postia* is supplied.

KEY WORDS — lignicolous, poroid, fungi, *Fomitopsidaceae*, taxonomy

Introduction

Postia Fr. (*Polyporales*, *Basidiomycota*) is an important genus of brown-rot fungi that are widely distributed in the northern hemisphere and mostly grow on gymnosperm wood. Some species of the genus are economically important, such as *Postia guttulata* (Peck) Jülich and *P. lactea* (Fr.) P. Karst., which have been used as medicine in China (Dai et al. 2009a).

Some mycologists consider *Postia* a synonym of *Oligoporus* Bref. (Gilbertson & Ryvarden 1987, Núñez & Ryvarden 2001, Ryvarden & Gilbertson 1994), while others regard it as an independent genus (Dai & Hattori 2007, Larsen & Lombard 1986, Niemelä et al. 2004, Renvall 1992, Wei & Dai 2006). The genus is characterized by an annual growth habit, a monomitic hyphal system with clamp connections, and thin-walled, allantoid to cylindrical or ellipsoid basidiospores. Twenty-eight species have previously been known from China (Cui et al. 2008, Dai 2009, Dai et al., 2003, 2004, 2007a,b, 2009b, Dai & Penttilä 2006, Li et al. 2007, 2008, Wang et al. 2009, 2011, Wei & Dai 2006, Wei & Qin 2010, Yuan & Dai 2008, Yuan et al. 2010).

During a study on the taxonomy and diversity of wood-rotting fungi in Northeast China, two *Postia* specimens were found that represented one previously undescribed species, which we propose as *P. subplacenta*. In addition, we provide an identification key to the *Postia* species thus far reported from China.

Materials & methods

The studied specimens were deposited in the herbarium of Beijing Forestry University (BJFC). The microscopic procedure follows Dai (2010) and Cui et al. (2011). In presenting the variation in the size of the spores, we exclude 5% of measurements (given in parentheses) from each end of the range. The following abbreviations are used: IKI = Melzer's reagent, IKI- = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, n = number of spores measured from given number of specimens. Sections were studied at magnifications up to $\times 1000$ using a Nikon Eclipse E 80i microscope and phase contrast illumination. Drawings were made with the aid of a drawing tube. Special colour terms follow Petersen (1996).

Taxonomy

Postia subplacenta B.K. Cui, sp. nov.

FIG. 1

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Differs from *Postia placenta* in the cream-buff to pale cinnamon-buff pore surface, wider subicular hyphae, smaller basidiospores, and absence of gloeopleurous hyphae.

TYPE. — China. Jilin Province, Antu County, Changbaishan Nature Reserve, on fallen trunk of *Pinus*, 8.VIII.2011, Cui 10001 (holotype, BJFC).

ETYMOLOGY — *subplacenta* (Lat.) denotes a similarity with *Postia placenta*.

FRUITBODY — Basidiocarps annual, resupinate, corky when fresh, corky to fragile when dry, up to 5 cm long, 3 cm wide, and 2.5 mm thick at the center. Pore surface white to cream-buff when fresh, cream-buff to pale cinnamon-buff when dry; pores angular, 3–5 per mm; dissepiments thin, entire to lacerate. Margin indistinct, narrow to almost lacking. Subiculum cream to cream-buff, corky when dry, ≤ 0.3 mm thick. Tubes paler than pore surface, pale cream-buff, fragile when dry, ≤ 2.2 mm long.

HYPHAL STRUCTURE — Hyphal system monomitic; generative hyphae bearing clamp connections, IKI-, CB-; tissues unchanged in KOH.

SUBICULUM — Generative hyphae hyaline, thin- to mostly slightly thick-walled, occasionally branched, interwoven, 3–7 μm in diam.

TUBES — Generative hyphae hyaline, slightly thick- to distinctly thick-walled, with a wide to narrow lumen, occasionally branched, interwoven, 2–5 μm in diam. Cystidia and cystidioles absent. Basidia clavate with four sterigmata and a basal clamp connection, 12–18 $\times$ 4–5 μm ; basidioles in shape similar to basidia, but slightly smaller.

SPORES — Basidiospores cylindrical, hyaline, thin-walled, smooth, IKI-, CB-, (4.0–)4.2–6.0(–6.9) $\times$ 1.9–2.4(–2.5) μm , L = 5.07 μm , W = 2.11 μm , Q = 2.37–2.45 (n = 60/2).

TYPE OF ROT — Brown rot.

ADDITIONAL SPECIMEN EXAMINED — CHINA. HEILONGJIANG PROVINCE, YICHUN, Fenglin Nature Reserve, on stump of *Pinus*, 1.VIII.2011 Cui 9818 (BJFC).

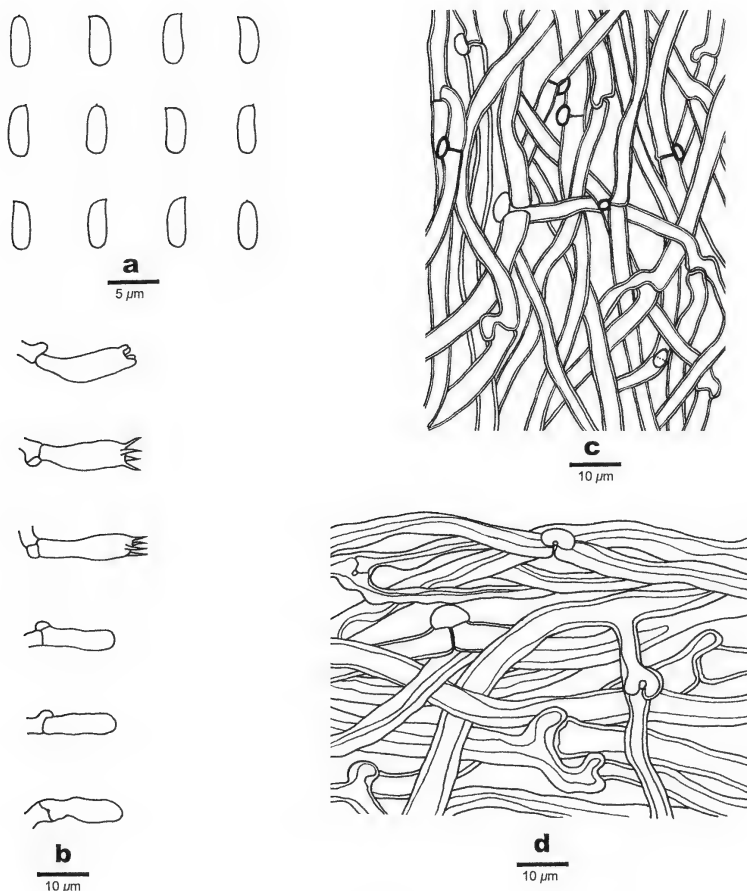


FIG. 1. *Postia subplacenta* (holotype), microscopic structures.

a: Basidiospores. b: Basidia and basidioles. c: Hyphae from trama. d: Hyphae from subiculum.

REMARKS — *Postia subplacenta* resembles *P. placenta* (Fr.) M.J. Larsen & Lombard in having resupinate basidiocarps, similar pores, and cylindrical basidiospores; however, *P. placenta* has a salmon-pink pore surface, gloeopleurous hyphae, narrow subicular hyphae, and larger basidiospores ($5.5\text{--}7 \times 2\text{--}2.5 \mu\text{m}$; Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994).

Postia subplacenta is similar to *P. rancida* (Bres.) M.J. Larsen & Lombard, which differs in having rancid taste and larger basidiospores ($6\text{--}8 \times 2\text{--}3 \mu\text{m}$; Gilbertson & Ryvarden 1987).

Postia obliqua Y.L. Wei & W.M. Qin, recently described from Southwest China (Wei & Qin 2010), may be confused with *P. subplacenta* in its resupinate

basidiocarps and similar pores and basidiospores ($4.8\text{--}6.3 \times 2.0\text{--}2.5\ \mu\text{m}$) but differs in its very large basidiocarps with oblique tubes that usually form imbricate pseudopilei and the presence of gloeopleurous-like hyphae.

Macroscopically, *Postia subplacenta* looks somewhat like *Oligoporus rennyi* (Berk. & Broome) Donk, which, however, has chlamydospores and produces basidiospores that are oblong-ellipsoid and thin- to slightly thick-walled (Ryvarden & Gilbertson 1994).

Key to species of *Postia* in China

1. Basidiocarps effused-reflexed, pileate or stipitate	2
1. Basidiocarps resupinate	23
2. Basidiocarps stipitate or substipitate	3
2. Basidiocarps effused-reflexed or pileate	4
3. Pores 1–3 per mm	<i>P. subundosa</i> Y.L. Wei & Y.C. Dai
3. Pores 3–5 per mm	<i>P. ceriflua</i> (Berk. & M.A. Curtis) Jülich
4. Basidiocarps with distinct grey to bluish tints	5
4. Basidiocarps white, cream, yellowish or brown	7
5. On angiosperm	<i>P. alni</i> Niemelä & Vampola
5. On gymnosperm	6
6. Basidiospores $<1.8\ \mu\text{m}$ wide	<i>P. caesia</i> (Schrad.) P. Karst.
6. Basidiospores $>1.8\ \mu\text{m}$ wide	<i>P. luteocaesia</i> (A. David) Jülich
7. Basidiocarps becoming brown when bruised or when dry	8
7. Basidiocarps unchanged when bruised or dry	10
8. Gloeocystidia present	<i>P. leucomallella</i> (Murrill) Jülich
8. Gloeocystidia absent	9
9. Basidiospores $<1.6\ \mu\text{m}$ wide	<i>P. lateritia</i> Renvall
9. Basidiospores $>1.7\ \mu\text{m}$ wide	<i>P. fragilis</i> (Fr.) Jülich
10. Basidiocarps chalky when dry	<i>P. calcarea</i> Y.L. Wei & Y.C. Dai
10. Basidiocarps fragile, corky or woody hard when dry	11
11. Cystidia present	12
11. Cystidia absent	14
12. Cystidia thin-walled	<i>P. amurensis</i> Y.C. Dai & Penttilä
12. Cystidia thick-walled	13
13. Cystidia amyloid in Melzer's reagent	<i>P. pileata</i> (Parmasto) Y.C. Dai & Renvall
13. Cystidia inamyloid in Melzer's reagent	<i>P. balsamea</i> (Peck) Jülich
14. Pores bigger, 1–3 per mm	<i>P. undosa</i> (Peck) Jülich
14. Pores smaller, >3 per mm	15

15. Gloeocystidia present	16
15. Gloeocystidia absent	17
16. Hyphal pegs abundant	<i>P. gloeocystidiata</i> Y.L. Wei & Y.C. Dai
16. Hyphal pegs absent	<i>P. qinensis</i> Y.C. Dai & Y.L. Wei
17. Pileal surface more or less pink when fresh	18
17. Pileal surface never pink when fresh	19
18. Basidiospores >1.5 µm wide	<i>P. persicina</i> Niemelä & Y.C. Dai
18. Basidiospores <1.5 µm wide	<i>P. cana</i> H.S. Yuan & Y.C. Dai
19. Pileal surface zonate	<i>P. zebra</i> Y.L. Wei & Y.C. Dai
19. Pileal surface azonate	20
20. Basidiocarps mild, upper surface greyish brown	<i>P. tephroleuca</i> (Fr.) Jülich
20. Basidiocarps bitter, upper surface cream or yellow-brownish	21
21. Basidiospores >2 µm wide	<i>P. guttulata</i>
21. Basidiospores <2 µm wide	22
22. Basidiocarps woody hard when dry	<i>P. stiptica</i> (Pers.) Jülich
22. Basidiocarps fragile when dry	<i>P. lactea</i>
23. Basidiocarps becoming reddish to rusty brown when touched	<i>P. lateritia</i>
23. Basidiocarps unchanged when touched	24
24. Basidiospores mostly >2 µm wide, cystidia absent	25
24. Basidiospores mostly <2 µm wide, cystidia present	28
25. Basidiocarps salmon pink; basidiospores 2–3 µm wide	<i>P. placenta</i>
25. Basidiocarps white or cream; basidiospores 2–2.5 µm wide	26
26. Basidiocarps with rancid smell	<i>P. rancida</i>
26. Basidiocarps without rancid smell	27
27. Basidiocarps very large, gloeopleurous hyphae present	<i>P. obliqua</i>
27. Basidiocarps small, gloeopleurous hyphae absent	<i>P. subplacenta</i>
28. Cystidia thick-walled	<i>P. pileata</i>
28. Cystidia thin-walled	29
29. Pores 5–6 per mm; basidiospores mostly <1 µm wide	<i>P. simanii</i> (Pilát) Jülich
29. Pores 3–4 per mm; basidiospores mostly >1 µm wide	<i>P. hibernica</i> (Berk. & Broome) Jülich

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MYCOTAXON

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Three new *Caeoma* species on *Rosa* spp. from PakistanN.S. AFSHAN^{1*}, A.N. KHALID² & A.R. NIAZI²¹*Centre for Undergraduate Studies & ²Department of Botany, University of the Punjab,
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ABSTRACT — Three representatives of the anamorphic genus *Caeoma* — *C. ahmadii* on *Rosa microphylla*; *C. khanspurens* and *C. rosicola* on *Rosa webbiana* — are described as new rust species from Pakistan. This first report of *Caeoma* raises the number of known anamorphic rust genera from the country to five.

KEY WORDS — Khanspur, Mansehra, *Phragmidium*

Introduction

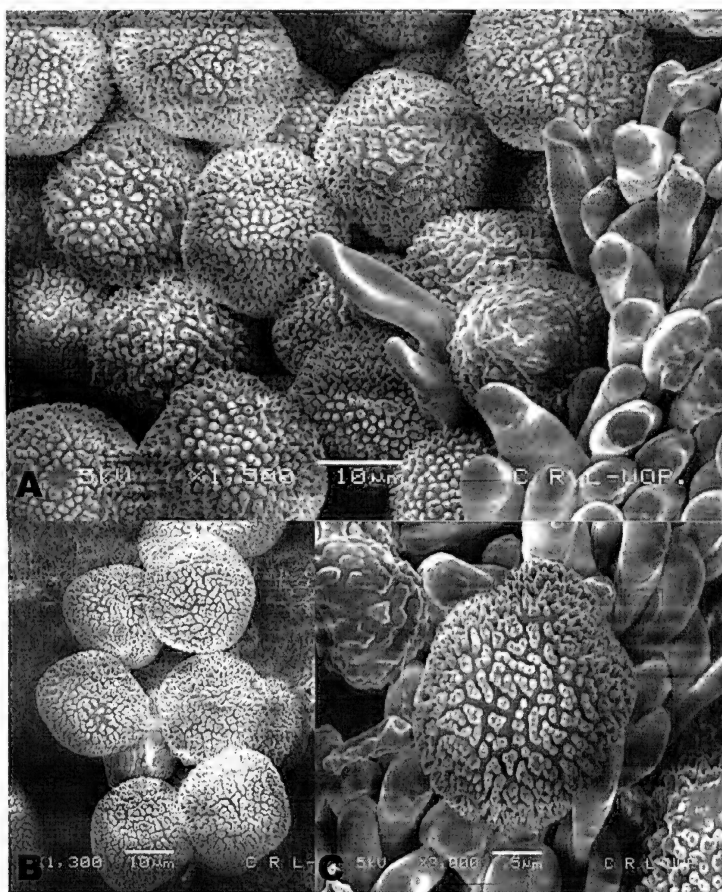
The genus *Caeoma* Link is traditionally used for species having sori that lack obvious bounding structures and that produce catenulate spores with intercalary cells. This contrasts with the genus *Aecidium* Pers., which has a cup-shaped sorus with a well-developed peridium. Similar sori are found in the aecial state of *Melampsora* Castagne and the uredinia of *Chrysomyxa* Unger, *Coleosporium* Lév., and other genera (Cummins & Hiratsuka 2003). The aecia of *Phragmidium* are (usually) *Caeoma*-type with catenulate spores or (less often) *Uredo*-type (Petrova & Denchev 2004) with verrucose or echinulate aeciospores. Cummins & Hiratsuka (2003) refer to the anamorphic genus *Lecythea* Léveillé the *Phragmidium* species with aecia corresponding to *Caeoma* III of Sato & Sato (1985). Because Hennen et al. (2005) regard *Lecythea* as confusing and not in use, we consider *Caeoma* the appropriate anamorph for accommodating species with *Phragmidium* aecia.

So far, 22 genera and 417 species of rust fungi have been described or reported from Pakistan (Afshan et al. 2008a,b,c,d, 2009a,b, 2010a,b, 2011a,b; Sultan et al. 2008; Afshan & Khalid 2009; Khalid & Afshan 2009; Iqbal et al. 2008, 2009), including four genera of anamorphic rust fungi, *Aecidium*, *Monosporidium* Barclay, *Peridermium* (Link) J.C. Schmidt & Kunze, and *Uredo* Pers. Although no representatives of *Caeoma* have been reported previously from Pakistan, three new *Caeoma* species occurring on *Rosa* are described here.

Materials & methods

Freehand sections of infected tissue and spores were mounted in lactophenol and gently heated to boiling point. The preparations were observed under a NIKON YS 100 microscope and photographed with a digipro-Labomed and a JSM5910 scanning electron microscope. Drawings of spores and paraphyses were made using a Camera Lucida (Ernst Leitz Wetzlar, Germany). An ocular micrometer was used for spore dimensions, with at least 25 spores measured for each spore stage. The rusted specimens have been deposited in the Botany Department herbarium, University of the Punjab, Lahore (LAH).

Taxonomy



FIGS. A–C: *Caeoma ahmadii* (holotype), SEM micrographs. A: Acedidium containing aeciospores and paraphyses. B: Aeciospores. C: Detail of an aeciospore showing verrucose wall ornamentation (paraphyses visible in background).

Caeoma ahmadii Afshan, Khalid & Niazi, sp. nov.

FIGS. A–E

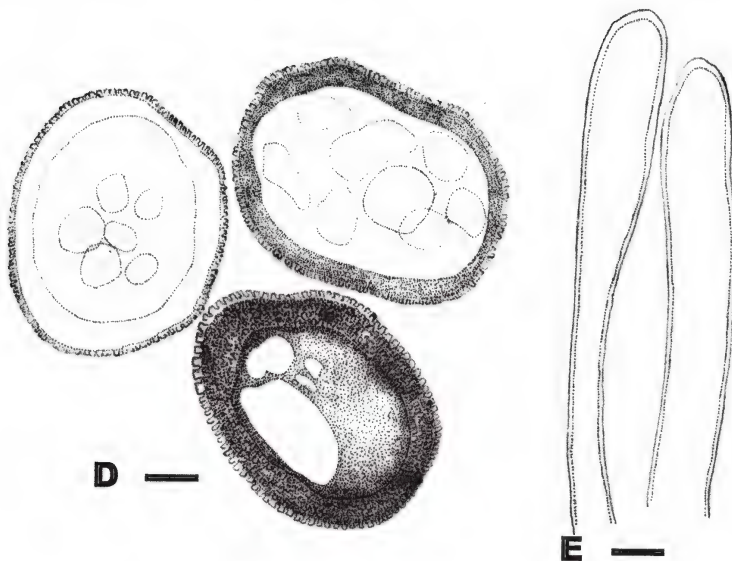
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Differs from *Phragmidium tuberculatum* in larger aeciospores that are hyaline with orange-yellow contents.

TYPE: PAKISTAN, KHYBER-PAKHTUNKHAWAH (KPK), Ayubia National Park, at 2135 m a.s.l., on *Rosa microphylla* Desf., stage I, 18 June, 2008, N.S. Afshan & A.N. Khalid. NSA #151A (Holotype, LAH NSA1114).

ETYMOLOGY: Named after the world renowned mycologist, Sultan Ahmad.

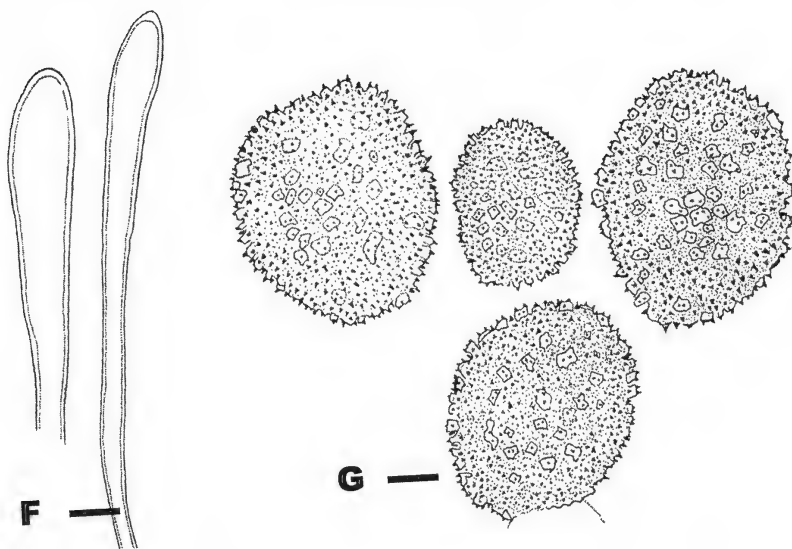
SPERMOGONIA, UREDINIA and TELIA not found. AECIA hypophyllous, on leaves, bright yellow to orangish yellow, rounded or oblong, scattered, 0.1–0.3 × 0.09–0.10 mm. PARAPHYSES numerous, erect to suberect, clavate, hyaline with light yellow granules, 50–70 µm long and 8–12 µm wide. AECIOSPORES globose to subglobose or ellipsoid to broadly ellipsoid, (23–)26–31 × 28–36 µm; wall 1–1.5 µm thick, hyaline with orange–yellow contents, densely verrucose, with 5–8 scattered pores.



FIGS. D–E: *Caeoma ahmadii* (holotype), lucida drawings.
D: Aeciospores showing wall ornamentation. Scale bar = 9 µm.
E: Apices of paraphyses. Scale bar = 12 µm.

COMMENTS: Aeciospores of *C. ahmadii* are larger than those of *Phragmidium tuberculatum* J.B. Müll. (18–24 × 20–30 µm), *Ph. mucronatum* (Pers.) Schtdl. (17–21 × 20–28 µm), *Ph. montivagum* Arthur (16–19 × 21–26 µm), *Ph. rosae-arkansanae* Dietel (16–23 × 23–27 µm), *Ph. speciosum* (Fr.) Burrill (16–24 ×

24–35 μm), and *Ph. rosae-pimpinellifoliae* Dietel (15–20 $\times$ 18–27 μm). They also differ in color and wall ornamentation from those of *Ph. tuberculatum*.



FIGS. F–G: *Caeoma khanspurens* (holotype), lucida drawings.
F: Apices of clavate paraphyses. Scale bar = 20 μm . G: Mature aeciospores. Scale bar = 10 μm .

***Caeoma khanspurens* Khalid, Afshan & Niazi, sp. nov.**

FIGS. F–G

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Differs from *Phragmidium mucronatum* and *Ph. tuberculatum* in larger aeciospores with verrucose wall ornamentation.

TYPE: PAKISTAN, KHYBER-PAKHTUNKHAWAH (KPK), Helipad, Khanspur, at 2135 m a.s.l., on *Rosa webbiana* Wall. ex Royle, stage I, 23 May 2006, N.S. Afshan & A.N. Khalid. NSA #03 (Holotype, LAH NSA1116).

ETYMOLOGY: Named after the type locality, Khanspur.

SPERMOGONIA, UREDINIA and TELIA not found. AECIA hypophyllous or cauliculous, on the veins, stems and petioles, scattered, rounded or oblong, 0.3–0.9 $\times$ 0.09–0.10 mm, orange yellow when fresh, surrounded by clavate paraphyses. AECIOSPORES globose, subglobose or ellipsoid to obovoid, pale yellow to orange-yellow, verrucose, occasionally with a plateau-shaped base, 23–32 $\times$ 23–41 μm , borne on one or two basal cells. PARAPHYSES clavate, hyaline, 70–105 μm long, 8–12 μm wide, with uniformly thin walls.

COMMENTS: Aeciospores of *C. khanspurens* are larger than those of *Phragmidium mucronatum* (17–21 $\times$ 20–28 μm) and *Ph. tuberculatum* (18–24

$\times 20\text{--}30\text{ }\mu\text{m}$); their verrucose ornamentation further distinguishes them from the aeciospores of *Ph. mucronatum*.

The broader aeciospores of *C. khanspurens* differ from those of *C. rosicola* (reported on the same host), characterized by verrucose to echinulate, $19\text{--}28\text{ }\mu\text{m}$ broad aeciospores.

Aeciospores with a verrucose wall ornamentation with a plateau-shaped base distinguish *C. khanspurens* from *Phragmidium rosae-pimpinellifoliae* with smaller aeciospores ($15\text{--}20 \times 18\text{--}27\text{ }\mu\text{m}$).

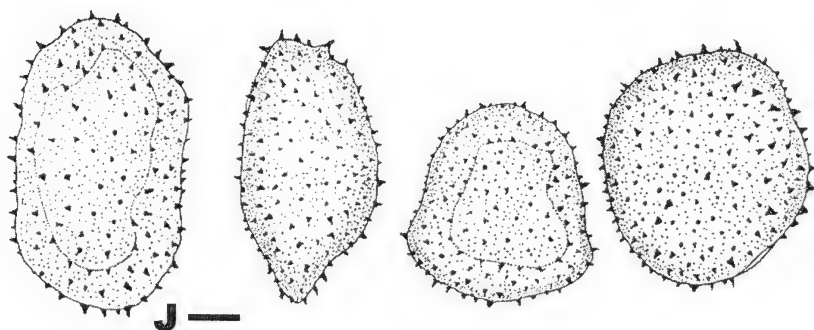


FIG. H: *Caeoma rosicola*, lucida drawing.
Aeciospores. Scale bar = $10\text{ }\mu\text{m}$.

Caeoma rosicola Afshan, Niazi & Khalid, **sp. nov.**

FIGS. H–J

MYCOBANK MB 564318

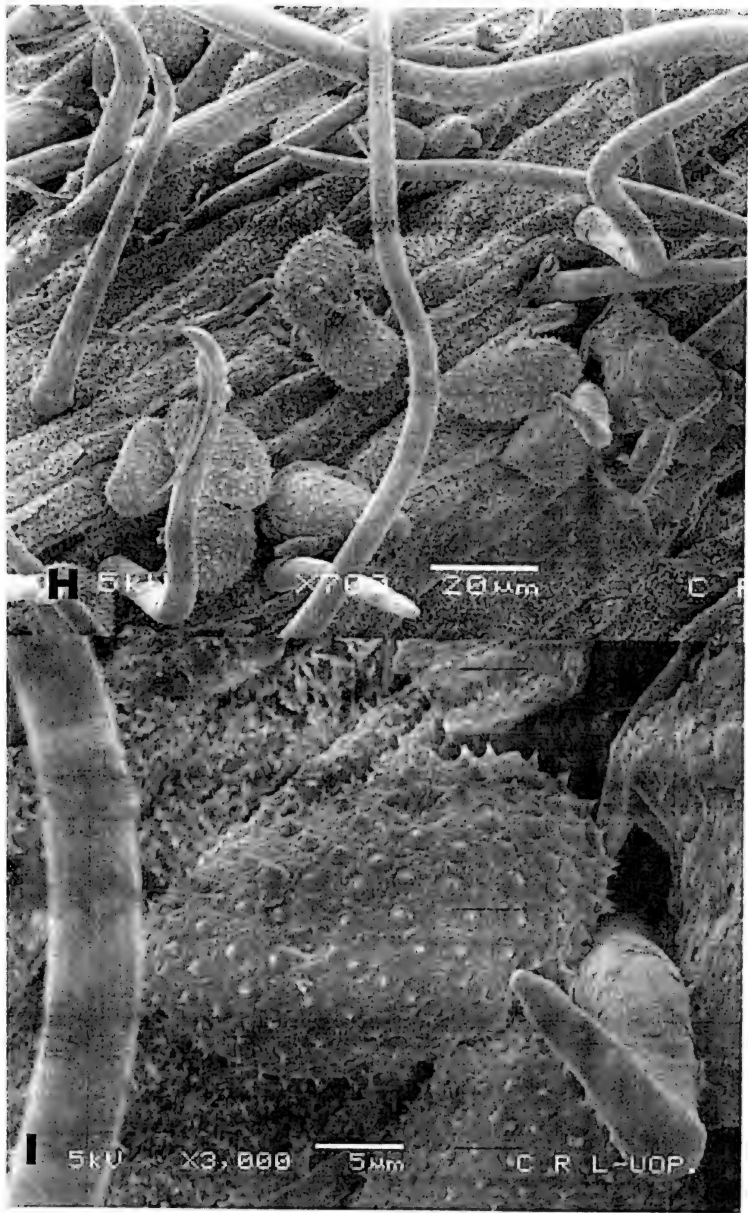
Differs from other *Caeoma* species in larger aeciospores and lack of paraphyses.

TYPE: PAKISTAN, KHYBER-PAKHTUNKHAWAH (KPK), Helipad, Khanspur, at 2135 m a.s.l., on *Rosa webbiana* Wall. ex Royle, stage I, 23 May 2006, N.S. Afshan & A.N. Khalid. NSA #02 (Holotype, LAH NSA1117).

ETYMOLOGY: Named after the host genus, *Rosa*.

SPERMOGONIA, UREDINIA and TELIA not found. AECIA hypophyllous or petiolicolous, mostly on the veins, petioles, branches and fruits, causing malformations, yellowish orange to bright orange, scattered. PARAPHYSES not observed. AECIOSPORES globose to subglobose or ellipsoid to ovoid; wall hyaline with orange yellow contents, echinulate to verrucose, $19\text{--}28 \times 21\text{--}41\text{ }\mu\text{m}$, germ pores 1–4, mostly equatorial.

COMMENTS: Larger aeciospores and the lack of paraphyses differentiate *C. rosicola* from other rust species reported on *Rosaceae*: *Phragmidium rosae-pimpinellifoliae* ($15\text{--}20 \times 18\text{--}27\text{ }\mu\text{m}$), *Ph. rosae-arkansanae* ($16\text{--}23 \times 23\text{--}27\text{ }\mu\text{m}$),



FIGS. I–J: *Caeoma rosicola* (holotype), SEM micrographs. I: Aeciospores. J: Detail of aeciospore showing echinulate wall ornamentation.

Ph. mucronatum (17–21 × 20–28 µm), and *Ph. montivagum* (16–19 × 21–26 µm). *Phragmidium montivagum* is further distinguished by aeciospores with 8 scattered germ pores.

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**Nomenclatural status and morphological notes on
Tubifera applanata sp. nov. (Myxomycetes)**D.V. LEONTYEV^{1*} & K.A. FEFELOV²¹*Department of Biotechnology, Kharkiv Zooveterinary Academy,
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ABSTRACT — *Tubifera applanata* sp. nov. is proposed to validate “*Tubulifera applanata*” nom. inval. The species diagnosis and some notes on its morphology are provided. At 0.40–0.65 mm diam., individual sporothecae are somewhat larger than in *T. ferruginosa* and *T. microsperma* and smaller than those in *T. casparyi*. Circular ornamentations on the inner peridial surface in *T. applanata* are larger than previously noted, reaching a size up to 2.9 µm.

KEY WORDS — morphometry, SEM, species delimitation

Introduction

In 2001, C. Lado stated that the invalid generic name “*Tubulifera*” O.F. Müll. 1775 had been validated as *Tubulifera* O.F. Müll. ex Jacq. 1779, a name with priority over *Tubifera* J.F. Gmel. 1792 (Lado 2001). On this understanding, Leontyev & Fefelov (2009) proposed a new species, *Tubulifera applanata*.

Subsequently, Lado (2011) indicated that, contrary to previous opinion, Jacquin’s publication failed to validate the genus “*Tubulifera*.” This means that all species names published in “*Tubulifera*,” including “*Tubulifera applanata*,” are also invalid (ICBN [Vienna Code] Article 43.1). We therefore propose a validation of “*Tubulifera applanata*” as *Tubifera applanata* sp. nov. and provide additional morphological data.

Materials & methods

A total of 13 specimens of *Tubifera applanata* were examined (CWU MR 001, 038a, 038b, 039, 054, 058, 074, 077, 121, 122, 123, 125, 126). Additionally, 8 specimens of *T. ferruginosa* (Batsch) J.F. Gmel. (CWU MR 006, 023, 012, 091, 095, 103, 113, 114), 4 of *T. microsperma* (Berk. & M.A. Curtis) G.W. Martin (CWU MR 008, 041, 049, 153), and 3 of *T. casparyi* (Rostaf.) T. Macbr. (CWU MR 049, 181, 183) were used for comparison. All the specimens were collected during 2003–2010 in the East Forest-Steppe region

of Ukraine (Gomolsha Forests National Nature Park, Kharkiv region). Material was deposited in the herbarium of the V.N. Karasin National University of Kharkiv (CWU), the subsection of Reticulariaceae (MR).

The specimens were examined using a stereoscopic microscope MBS-9 and scanning electron microscope (SEM) Jeol JSM-6060 (30 kV, gold evaporation).

The diameters of intact closed sporothecae were measured from above by a micrometer. After drawing an imaginary line corresponding to the largest fruit body diameter on the surface of the pseudoaethalium, we measured 30 sporothecae along this line from the edge of the pseudoaethalium. The diameters of annular ornamentations on the inner peridial surface were measured using the SEM scale.

Means, standard deviations, and standard errors were calculated using Stat Soft Statistica 8.0 and Microsoft Excel 2003 software to describe the morphological variety.

Results & discussion

Tubifera applanata Leontyev & Fefelov, sp. nov.

FIG. 1C–D

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"*Tubulifera applanata*" Leontyev & Fefelov, Bol. Soc. Mycol. Madrid 33: 119. 2009

Differs from *Tubifera ferruginosa* by its large pseudoaethalia, flat sporothecal tips, annular embossments on the internal peridial surface, and salmon color of young fructifications.

TYPE: Ukraine, Kharkiv region, Gomolsha Forests National Nature Park, Zadinetske forestry, edge of pine forest near 'Bile Ozero' Camp (49°36'50.69"N 36°21'06.15"E), on the bark of fallen trunk of *Pinus sylvestris* L., 14.07.2003, leg. D.V. Leontyev (*Holotype*, CWU MR 039).

ETYMOLOGY: *applanatus* (Lat.) = flat; referring to the flattened sporothecal tips.

PSEUDOAEATHALIUM large, (12–)23–37(–73) mm long, (7–)16.5–27.5(–38) mm wide, 4–6 mm in height, oval or irregular as seen from above, flat pulvinate, rust-brown. SPOROTHECAE straight, clearly prismatic from mutual pressure. TIPS OF SPOROTHECAE 0.4–0.65 mm diam., flat or slightly convex, 5–6 angular or almost circular, equal in height, forming a smooth surface of pseudoaethalium. PERIDIUM opaque, light brown, lateral walls of the sporothecae often plicate. INTERNAL SURFACE OF PERIDIUM covered with annular embossments (0.4–)1.5–2.9 µm diam., quite distant from each other. COLUMELLA absent or not reaching the top of the sporothecae, thick, with color of spore mass. PSEUDOCAPILLITIUM absent or scanty, filiform, appearing as occasionally branched strings. HYPOTHALLUS spongy, poorly developed or absent. SPORES in mass rust-brown, brownish by transmitted light, 4.9–6.3(–7.5) µm diam., banded reticulate. PLASMIDIUM salmon or rusty-cream. In coniferous forests, on bark, litter and slightly decomposed wood.

Notes on the morphology

Tubifera applanata is characterized by the prismatic shape of the individual sporothecae, their flat hexagonal tips, ring-like ornamentations on the inner peridial surface, and the salmon color of young fructifications. Although we

first noted that quantitative parameters such as pseudoaethalium size and spore diameter distinguished *T. applanata* from related species (Leontyev & Fefelov 2009), we did not realize that individual sporothecal diameters might also serve as a diagnostic criterion. Moreover, there were no data referring to the diameter of sporothecae in other *Tubifera* species (Nannenga-Bremekamp 1991, Neubert et al. 1993, Ing 1999).

The study of sporothecae size in *Tubifera applanata*, *T. ferruginosa*, *T. microsperma*, and *T. casparyi* has shown that all species have significant differences ($p < 0.01$) from one another. Measurements show that individual sporothecae are somewhat larger in *T. applanata* than in *T. ferruginosa* and *T. microsperma* and smaller than those in *T. casparyi* (TABLE 1).

TABLE 1. Sporothecal diameters in four *Tubifera* species.

	<i>T. applanata</i>	<i>T. ferruginosa</i>	<i>T. microsperma</i>	<i>T. casparyi</i>
Range of specimen means (μm)	464–633	322–474	330–398	512–692
Mean (μm)	515	398	374	574
Standard deviation [sd] (μm)	116.0	95.2	67.6	98.2
Mean $\pm$ sd (μm)	399–631	302–493	306–442	476–672
Rounded variation, for use in species descriptions (mm)	0.4–0.65	0.3–0.5	0.3–0.45	0.5–0.7

Although the difference in sporothecae size between *T. applanata* and *T. ferruginosa* is quite small (0.1–0.2 mm), it can be seen with the naked eye. We propose to use this criterion as an additional character in descriptions of *Tubifera* species.

Peridium ultrastructure

Nelson et al. (1982) introduced a new diagnostic criterion into *Tubifera* taxonomy — the type of inner peridial ornamentation, noting that in *T. microsperma* the inner surface of the peridium is ornamented by so-called ‘rimmed craters.’ In Ukrainian specimens of *T. microsperma*, the ornamentation agrees with this description (FIG. 1A–B).

In 2009 the SEM-study of *T. applanata* showed ‘peridial rings,’ similar to those of *T. microsperma* but much lower and more distant from each other (Leontyev & Fefelov 2009). Both ‘peridial rings’ and ‘rimmed craters’ appear to be remnants of channels used by the plasmodium for active water evacuation (R. McHugh, pers. comm.).

We previously indicated the outer ‘peridial ring’ diameter in *T. applanata* as 0.4–0.9 μm (Leontyev & Fefelov 2009). Improved SEM techniques demonstrate that in the type specimen, these structures reach 1.6–2.9 μm diam. (FIG. 1C–D), significantly larger than similar structures in *T. microsperma*. In *T. applanata*, rings $< 1 \mu\text{m}$ diam. are usually less prominent than the larger ones.

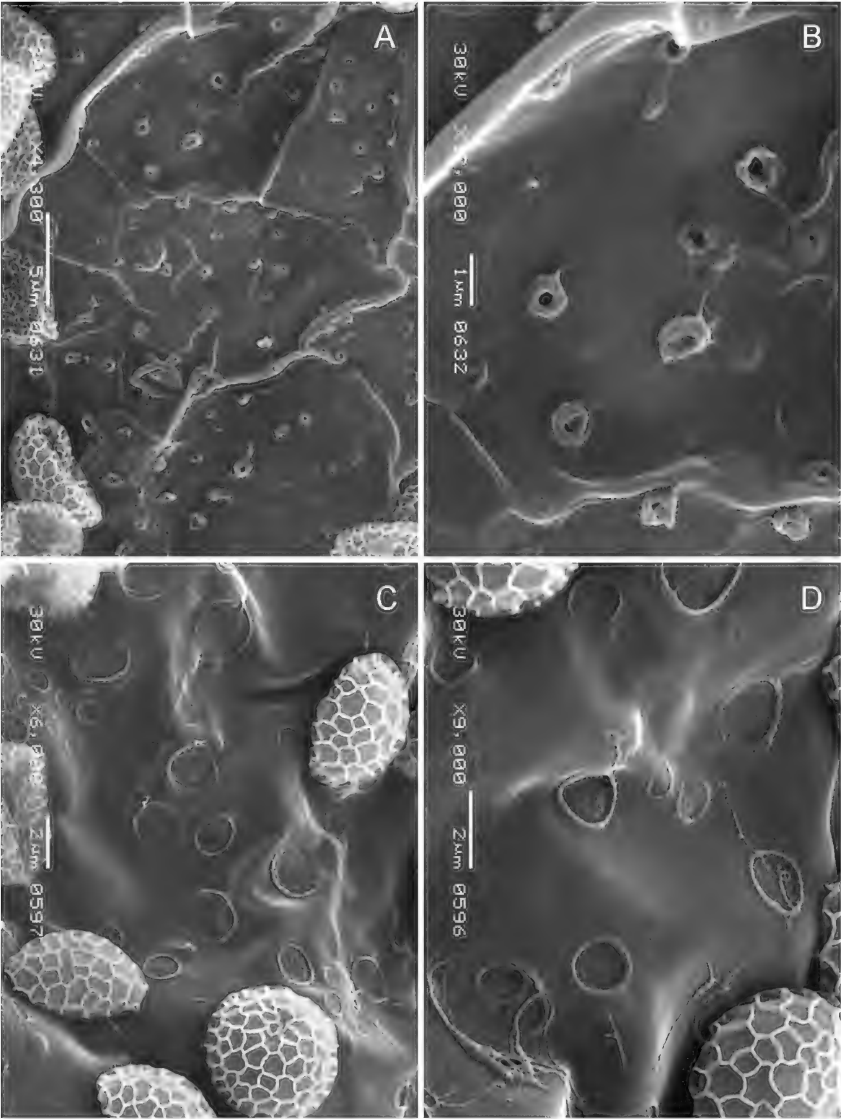


FIGURE 1. A–B: *Tubifera microsperma* [CWU MR 135]—ornamentation of the inner peridium surface illustrating ‘rimmed craters.’ C–D: *Tubifera applanata* [CWU MR 039, holotype]—ornamentation of the inner peridium surface illustrating ‘peridial rings’ and intricate filaments.

This study has shown that the peridial surface of *T. applanata* is ornamented by thin intricate filaments, appearing to be composed of dried slime (FIG. 1D). Such filaments are especially abundant in the bottom of the 'peridial rings.'

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***Paralepistopsis* gen. nov. and *Paralepista*
(Basidiomycota, Agaricales)**

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ABSTRACT — *Paralepistopsis*, a new genus in *Agaricales*, is proposed for the rare toxic species, *Clitocybe amoenolens* from North Africa (Morocco) and southern and southwestern Europe and *C. acromelalga* from Asia (Japan and South Korea). *Paralepistopsis* is distinguished from its allied clitocyboid genera by a *Lepista flaccida*-like habit, a pileipellis with diverticulate hyphae, small non-lacrymoid basidiospores with a smooth slightly cyanophilous and inamyloid wall, and the presence of toxic acromelic acids. Combined ITS-LSU sequence analyses place *Paralepistopsis* close to *Cleistocybe* and *Catathelasma* within the tricholomatoid clade. Our phylogenetic analysis further supports *Lepista* subg. *Paralepista* (= *Lepista* sect. *Gilva*) as an independent clitocyboid evolutionary line. We recognize the genus *Paralepista*, for which we propose twelve new combinations.

KEY WORDS — *Agaricomycetes*, erythromelalgia/acromelalgic syndrome, *Clitocybe* sect. *Gilva*oideae, *Catathelasma* clade

Introduction

The genus *Clitocybe* (Fr.) Staude traditionally encompassed saprobic agarics that produce fleshy basidiomata with often adnate-decurrent lamellae, convex to funnel-shaped pilei, usually a whitish to pinkish yellow spore print, and smooth non-amyloid basidiospores (Kühner 1980, Singer 1986, Bas 1990, Raithelhuber 1995, 2004).

Recent molecular studies that included a significant number of *Clitocybe* species (Moncalvo et al. 2002, Redhead et al. 2002, Matheny et al. 2006, Vizzini et al. 2010a,b, 2011) have shown that taxa in this traditional genus do not form a monophyletic group but rather a heterogeneous artificial set of disparate and (in many cases) phylogenetically unrelated taxa (the so-called clitocyboid fungi or *Clitocybe* s.l.).

Clitocybe amoenolens is a rare and rather localized species known thus far only from Morocco, southern France, northern and central Spain, and central

Italy (Malençon & Bertault 1975, Bon 1987, Poumarat & Neville 1993, Contu et al. 1999, Moreau et al. 2001, Martínez et al. 2010). It was responsible, first in France (Fourré 1997, Charignon & Garcin 1998, Moreau et al. 2001, Saviuc et al. 2001, 2002) and then in Italy (Leonardi et al. 2002, Marinetti & Recchia 2005), for induced erythromelalgia (= acromelalgia syndrome sensu Saviuc et al. 2001), a poisoning syndrome caused by the ingestion of *C. acromelalgia* in Japan (Nakamura et al. 1987). This syndrome is characterized by varying degrees of tingling sensations, followed by intense burning pain in the extremities but predominantly in the feet (Saviuc & Danel 2006). *Clitocybe amoenolens* was confused with edible mushrooms in the *Lepista flaccida* complex (e.g., *L. flaccida* (Sowerby) Pat., *L. lentiginosa* (Fr.) Bresinsky, *L. gilva* (Pers.) Pat.) and with *Infundibulicybe gibba* (Pers.) Harmaja (Fourré 1997, Moreau et al. 2001).

Clitocybe amoenolens shows features intermediate between *Clitocybe* s.s. (smooth spores) and *Lepista* subg. *Paralepista* (cream spore-print, spotted pileus, lamellae separable from context, and cyanophilic spores released in tetrads), making its generic position uncertain.

Using recent French and Italian collections of *C. amoenolens*, we investigated its phylogenetic position within the clitocyboid fungi through morphological and molecular analyses and expanded its known geographic distribution.

Materials & methods

Morphology

Macromorphological features were described from fresh specimens. Microscopical preparations from dried material were rehydrated in 3% KOH and stained in Congo red, Cresyl Blue, Cotton Blue and Melzer's reagent.

Basidiospore measurements are based on means of 120 spores from prints (four collections), stained in Melzer's reagent. The basidia width was measured at the widest part and the length from the apex (sterigmata excluded) to basal septum. The following abbreviations are used in text: L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae; Q = the quotient of length and width of the spores in side view; Qm = average quotient. Colour terms in capital letters (e.g. Pale Ochraceous-Buff) are those of Ridgway (1912). Herbarium acronyms follow Thiers (2011). Author citations follow Index Fungorum (<http://www.indexfungorum.org/authorsoffungalnames.htm>). All examined collections are housed at TO. The new genus and new combinations are deposited in MycoBank (<http://www.mycobank.org/DefaultPage.aspx>).

DNA extraction, PCR amplification, and DNA sequencing

Genomic DNA was isolated from 1 mg of herbarium material (TABLE 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy) according to the manufacturer's instructions. Universal primers ITS1F/ITS4 were used for the ITS region amplification (White et al. 1990, Gardes & Bruns 1993) and primers LR0R/LR7 for the LSU rDNA amplification (Vilgalys & Hester 1990, Vilgalys lab unpubl., <http://www.botany.duke.edu/fungi/>

TABLE 1. Collections newly sequenced in this study.

SPECIES	GENBANK ACC. NUMBERS		SOURCE, COUNTRY, DATE, COLLECTOR
	ITS	LSU	
<i>Paralepistopsis amoenolens</i> 1	JQ585653	JQ585654	TO AV2004, FRANCE, 02/09/2011, G. Moretto
<i>Paralepistopsis amoenolens</i> 2	JQ585655	—	TO AV2007, ITALY, 12/11/2011, S. Anselmino
<i>Paralepista flaccida</i> 1	JQ585656	JQ585657	TO AV2008, ITALY, 02/09/2011, G. Moretto
<i>Paralepista flaccida</i> 2	JQ585658	JQ585659	TO AV2009, ITALY, 20/10/2011, A. Vizzini
<i>Paralepista gilva</i>	JQ585660	JQ585661	TO AV2010, ITALY, 09/11/2011, A. Vizzini

mycolab). Amplification reactions were performed in a PE9700 thermal cyclers (Perkin-Elmer, Applied Biosystems) following Vizzini et al. (2010b). PCR products were purified with the AMPure XP kit (Beckman) and sequenced by DiNAMYCODE srl (Turin, Italy) and MACROGEN Inc. (Seoul, Republic of Korea). Sequences were assembled and edited with the phred/phrap/consed software suite. The sequences were submitted to GenBank and their accession numbers are reported in TABLE 1 and FIGURE 1.

Sequence alignment and phylogenetic analysis

The sequences obtained in this study were checked and assembled using Geneious v5.3 (Drummond et al. 2010) and compared to those available in GenBank (<http://www.ncbi.nlm.nih.gov/Genbank/>) using the blastn algorithm. Based on the blastn results, sequences were selected according to the outcomes of recent phylogenetic studies on *Agaricales* (Matheny et al. 2006, Binder et al. 2010, Vizzini et al. 2011). A combined ITS and LSU sequence analysis was carried out using sequences from the same strain or specimen. *Xeromphalina campanella* (GU320006 and GU320009) was used as outgroup. Alignments were generated using MAFFT (Katoh et al. 2002) with default conditions for gap openings and gap extension penalties. The sequence alignment, its manual adjustment, and the best-fit models estimation follow Vizzini et al. (2010b). The GTR+G and GTR+G substitution models were used in the ITS and LSU analyses, respectively. A partitioned matrix was used in all the analyses. Molecular-phylogenetic analyses were performed using the Bayesian Inference (BI) and Maximum Likelihood (ML) approaches. BI using Monte Carlo Markov Chains (MCMC) was carried out with MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). Four incrementally heated simultaneous MCMC were run over 5.000.000 generations, under model assumption. Trees were sampled every 500 generations resulting in an overall sampling of 10.001 trees. The “burn-in” value was evaluated using Tracer 1.5 (Rambaut & Drummond 2007). The first 20% of trees was discarded as “burn-in”. For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). ML estimation was performed through RAXML v.7.0.4 (Stamatakis 2006) with 1.000 bootstrap replicates (Felsenstein 1985) using the

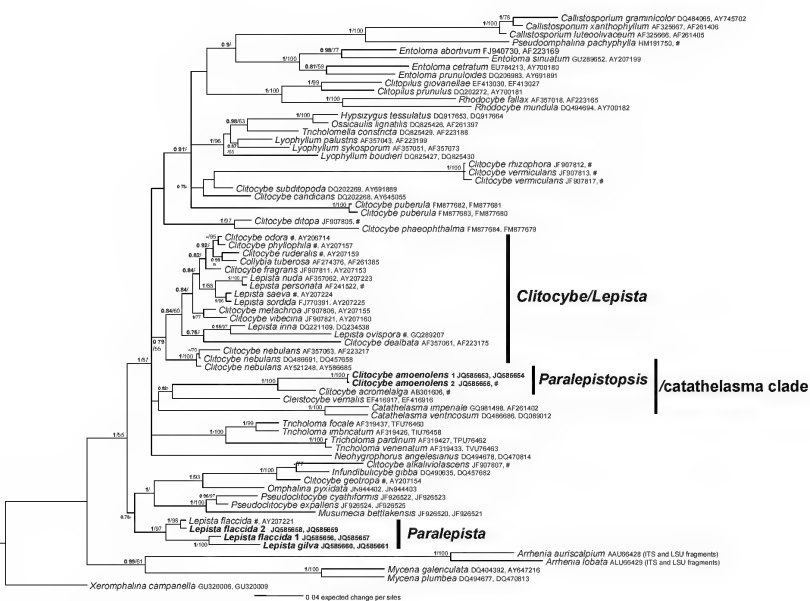


FIGURE 1. Tricholomatoid clade. Bayesian phylogram obtained from the combined ITS-LSU sequence alignment. Support values for clades that are supported in either the Bayesian (Posterior Probabilities values – BPP) or Maximum likelihood (ML Bootstrap percentage – MLB) analyses are indicated. BPP > 0.70 and MLB > 50% are given above branches. Numbers (1, 2) refer to the *Paralepistopsis* and *Paralepista* collections reported in TABLE 1.

GTRGAMMA algorithm for both ITS and LSU to perform a tree inference and search for a good topology. Support values from bootstrapping runs (MLB) were mapped on the globally best tree using the “-f a” option of RAXML and “-x 12345” as a random seed to invoke the novel rapid bootstrapping algorithm. Only BPP values over 0.70 and MLB over 50% are reported in the resulting tree (FIG. 1). Pairwise % identity values of ITS sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

Results

Molecular results

The combined dataset comprises a total of 71 taxa (including 66 from GenBank) and is 2412 base pairs long. The ITS and LSU datasets are 796 and 1616 base pairs long, respectively. Topologies of the combined ITS and LSU Bayesian and Maximum Likelihood trees are congruent (FIG. 1).

In both analyses the two *Clitocybe amoenolens* collections (ITS pairwise identity value = 99.9%) clearly cluster with the *C. acromelalga* collection (BPP 1 and MLB 100%) in the /catathelasma clade, a monophyletic group

in the tricholomatoid clade (Matheny et al. 2006, Ammirati et al. 2007). The ITS pairwise identity value between the *C. acromelalga* and *C. amoenolens* sequences is 94.4%; accepting an intraspecific variability lower than 3% (Nilsson et al. 2008), *C. amoenolens* and *C. acromelalga* should be considered distinct species. *Cleistocybe vernalis* Ammirati et al. cluster sister to the *C. acromelalga*/*C. amoenolens* pair (BPP 0.82); *Catathelasma* Lovejoy is basal to this group with low BPP and MLB values.

Clitocybe amoenolens and *C. acromelalga* have no phylogenetic relationship with other *Clitocybe* species. Accordingly, we propose to establish for them a new genus, *Paralepistopsis*.

Lepista flaccida and *L. gilva* are not phylogenetically related to the other *Lepista* species.

Taxonomy

Paralepistopsis Vizzini, gen. nov.

MYCOBANK MB 564340

A Paralepista differt sporis levis, haud subglobosis, atque praesentia acidi acromelalgici qua de causa venenatae species sunt. et in structura molecularis (ITS-spatii internis transcriptis et LSU DNA).

TYPE SPECIES — *Clitocybe amoenolens* Malençon

ETYMOLOGY — named in reference to its resemblance to *Paralepista* species.

Basidiomata agaricoid (with distinct pileus, lamellae and stipe), resembling those of *Paralepista gilva*, veils absent, spore-print whitish to cream, basidiospores thin-walled, smooth, inamyloid and slightly cyanophilous, pileal surface a cutis of repent to interwoven, cylindrical hyphae, clamp-connections present, no sarcodimitic texture in any part of the basidioma. On the ground, never on wood.

Paralepistopsis amoenolens (Malençon) Vizzini, comb. nov.

FIG. 2

MYCOBANK MB 564341

= *Clitocybe amoenolens* Malençon, in Malençon & Bertault, Flore des champignons supérieurs du Maroc 2 - Trav. Inst. Sci. Chérifien, Sér. Bot. Biol. Vég. 33: 141 (1975).

SELECTED DESCRIPTIONS — Malençon & Bertault (1975: 138–141); Moreau et al. (2001: 99–100, 101–103).

SELECTED ICONOGRAPHY — Malençon & Bertault (1975: pl. 8); Poumarat & Neville (1993: 48); Martínez et al. (2010: fig. 2, p. 104).

PILEUS (2–)3.5–7(–8) cm diam., fleshy, sub-elastic, hemispherical to convex at first, becoming plano-convex and applanate, finally plano-concave, at times broadly umbonate; margin narrowly inrolled and decurved at first, remaining inrolled for a long time, then expanding to become somewhat wavy, shortly sulcate-striate or corrugated; not hygrophanous, occasionally appearing

hygrophanous when water soaked; surface slightly viscid when moist, at first entirely whitely pruinose, then pubescent-pruinose only at margin, typically diffracted-scaly near the disc, sometimes corrugated, wrinkled and areolate, later subglabrous often with more or less concentrically arranged watery, drop-like spots, especially near margin (as many *Lepista* species); at first cream-beige coloured (Capucine Buff, Pale Ochraceous-Salmon, Pale Ochraceous-Buff), then pinkish beige to rusty orange (Ochraceous-Buff, Zinc Orange) towards the centre. LAMELLAE crowded to close ($L = 40\text{--}48(-50)$, $l = (0\text{--})1\text{--}2(-3)$), thick, interspersed with lamellulae, $(2\text{--})3\text{--}4(-5)$ mm broad, decurrent, easily separable from the pileus context, at times intervenose or forked towards the stipe, at first whitish then yellowish ochre (Ivory Yellow, Chamois) to pinkish beige (Pale Ochraceous-Salmon, Pale Ochraceous-Buff); edges even, entire, concolorous. STIPE $(2.5\text{--})3\text{--}3.5(-5)$ cm long, $0.7\text{--}1.3$ cm thick, short, central or subexcentric, equal or with a somewhat enlarged base, straight or recurved, concolorous with the pileus or slightly paler, minutely white pruinose at apex, glabrous elsewhere, stuffed with white medulla, becoming hollow; the base often with copious whitish tomentum with adhering *Pinaceae* needles and woody debris. CONTEXT $4\text{--}11(-14)$ mm thick at disc, elastic, white in the pileus, whitish cream (Capucine Buff) in the stipe cortex, unchanging; taste mild, fungoid, subfarinaceous, slightly bitter-farinaceous after long mastication; odour strong, aromatic, floral, reminiscent of *Inocybe corydalina*, *Tricholoma caligatum*, *Lepista irina*, or *Entoloma ameides*. SPORE PRINT whitish to pale cream (Light Buff, Pale Pinkish Buff).

BASIDIOSPORES $(3.8\text{--})4.0\text{--}5.4(-5.6) \times (2.3\text{--})3.2\text{--}4.0(-4.3)$ μm ($n = 120$), on average 4.8×3.4 μm , $Q = 1.3\text{--}1.7$, $Q_m = 1.42$, broadly ellipsoid, hyaline, thin-walled, smooth, slightly cyanophilous, inamyloid, non-dextrinoid, usually with only a single oil drop and a distinct truncated apiculus up to 0.7 μm long, mostly adhering together in tetrads in dried specimens. BASIDIA $(25\text{--})30\text{--}37(-38) \times 5\text{--}6(-7)$ μm , cylindro-clavate, usually four-spored, occasionally two-spored, sterigmata up to 5 μm long. HYMENOPHORAL TRAMA regular in young stages, but subirregular in mature basidiomata, consisting of hyaline, elongated, cylindrical hyphae $4\text{--}6(-8)$ μm broad. PLEUROCYSTIDIA absent. LAMELLA EDGES fertile, with rare, scattered cells, not well differentiated from basidia, $15\text{--}45(-60) \times 2.5\text{--}5(-7)$ μm , cylindric to subfusiform or sublageniform, often curved and flexuous, sometimes forked at apex, hyaline, thin-walled. PILEIPELLIS duplex: upper layer (suprapellis) a soon disappearing, slightly gelatinous thin cutis ($10\text{--}30$ μm thick), of cylindrical hyphae, $1\text{--}3$ μm broad; lower layer (subpellis) ($150\text{--}350$ μm thick) composed of densely arranged parallel to slightly interwoven hyphae ($4\text{--}6$ μm in diam.), terminal elements scattered, erect and repent cylindrical to fusiform-lageniform, sometimes with short lateral outgrowths. PILEITRAMA consisting of cylindrical or slightly

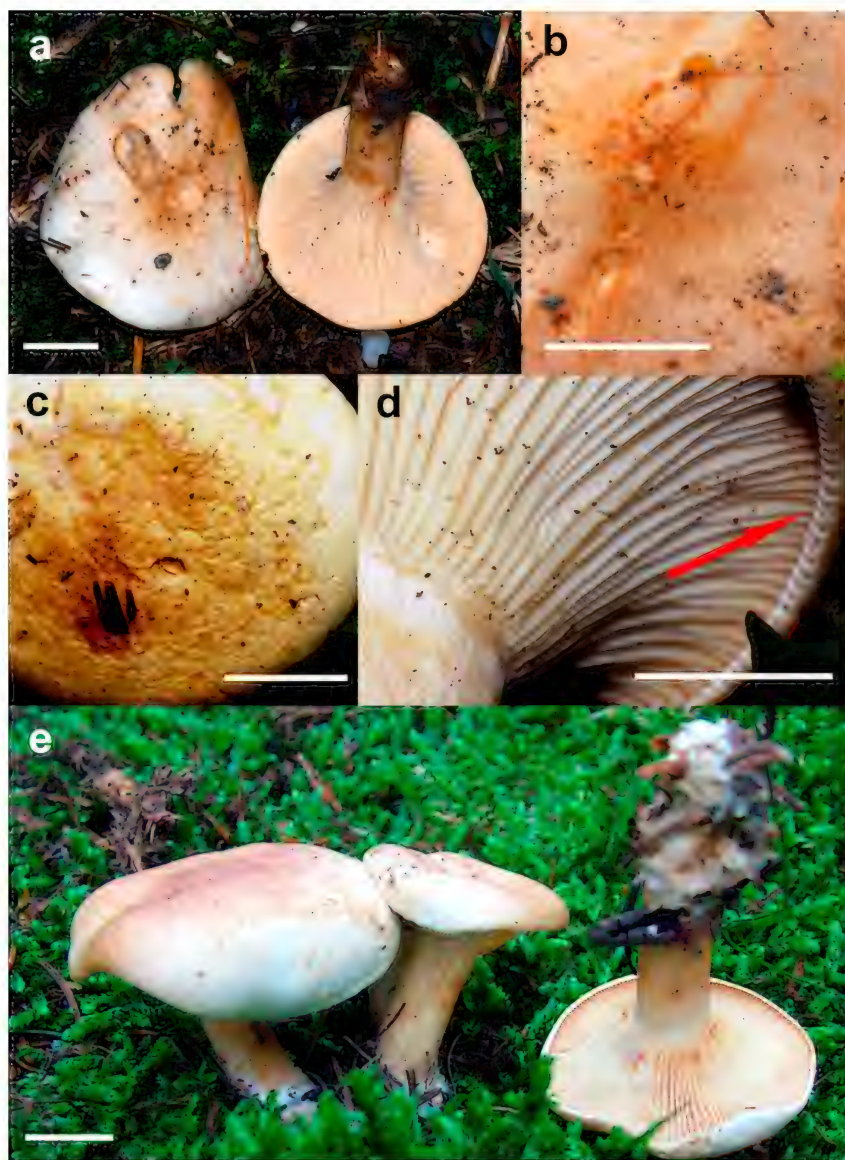


FIGURE 2. *Paralepistopsis amoenolens*. a. Basidiomata. b. Diffracted-scaly pileus with minute squamules. c. Areolate-corrugated pileus. d. Sulcate-striate pileus margin (red arrow), lamellae, and stipe. e. Basidiomata. [a–b from TO AV2007; c–d from TO AV2006; e from TO AV2004. Bars = 1 cm]

inflated, smooth hyphae, (4–)5–9(–12) μm broad, irregularly arranged, and with aeriferous lacunae. STIPITPELLIS a cutis consisting of hyphae 3–4 μm broad; towards the stipe apex with numerous thin-walled, flexuous, cylindrical to subfusiform caulocystidioid elements, $20\text{--}35 \times 3\text{--}6 \mu\text{m}$. STIPITITRAMA formed by hyphae 6–7 μm broad. THROMBOPLEROUS HYPHAE (= oleiferous hyphae sensu Cl  men  on 2004) present especially in subpellis, pileus and hymenophoral trama. PIGMENT (yellowish) usually parietal and intracellular (cytoplasmatic) in the pileipellis; sometimes minutely incrusting or forming extracellular masses and granules. CLAMP CONNECTIONS present at nearly all septa.

HABITAT & DISTRIBUTION: scattered, gregarious, occasionally subcaespitose on pineaceous needle-beds and debris, often together with *Lepista flaccida*, on calcareous soil. Autumn. Very rare; known only from Morocco, France, Spain, and Italy.

MATERIAL STUDIED: FRANCE, SAVOIE, HAUTE-MAURIENNE VALLEY, Lanslebourg-Mont-Cenis, 02 Sept 2011, litter of *Picea abies*, 1400 m asl, leg. G. Moretto (TO AV2004); 14 Oct 2011, leg. G. Moretto (TO AV2005). – ITALY, PIEDMONT, HIGH SUSA VALLEY, Salbertrand, Parco Naturale del Gran Bosco di Salbertrand, 20 Oct 2011, litter of *Picea abies*, 1500 m asl, leg. A. Vizzini (TO AV2006); Sauze d'Oulx, Parco Naturale del Gran Bosco di Salbertrand, 12 Nov 2011, litter of *Larix decidua*, 1550 m asl, leg. S. Anselmino (TO AV2007).

Clitocybe amoenolens was originally described from Morocco, in the Middle Atlas, growing among *Cedrus atlantica* litter in a high-altitude cedar forest (1600–1700 m a.s.l.) mixed with *Ilex aquifolium* and *Quercus ilex*, on calcareous soil (Malen  on & Bertault 1975). It has recently been found in southern France (Bon 1987, Poumarat & Neville 1993) and in the Maurienne Valley (Charignon & Garcin 1998, Fourr   1997) in coniferous forests (*Pinus sylvestris*, *Larix decidua*, *Picea abies*) and always on calcareous soil.

Clitocybe amoenolens has been responsible for several erythromelalgic-type poisonings in the Maurienne Valley (Savoie, France) (Moreau et al. 2001, Saviuc & Danel 2006). It also has been found in the Abruzzi region (Centre Italy) under *Pinus nigra* (Contu et al. 1999), *P. nigra*, and *Cedrus* spp. (Leonardi et al. 2002) and *P. nigra* and *Larix decidua* (Leonardi & Maggi 2007), and poisoning cases referable to *C. amoenolens* have been recognised in this region (Leonardi et al. 2002, Marinetti & Recchia 2005). Finally, the species has been reported from Spain (Mart  nez et al. 2010), where it was collected in the autonomous regions of La Rioja (*Picea abies*) and Castilla-La Mancha (*Pinus pinaster* and *Cupressus arizonica* or *P. nigra* and *Quercus petraea*), close to the north and centre of Spain, respectively.

Our collections are the first record from northern Italy. According to our observations and bibliographic data, this species seems strictly restricted to *Pinaceae* in higher altitude thermophilic forests on calcareous soils.

***Paralepistopsis acromelalga* (Ichimura) Vizzini, comb. nov.**

MYCOBANK MB564342

= *Clitocybe acromelalga* Ichimura, Bot. Gaz. (Tokyo) 65: 110 (1918).

SELECTED DESCRIPTIONS — Ichimura (1918: 110); Moreau et al. (2001: 109–111).

SELECTED ICONOGRAPHY — Guez (2010: (<http://www.mycodb.fr/fiche.php?genre=Clitocybe&espece=acromelalga>)).

HABITAT & DISTRIBUTION: *Clitocybe acromelalga*, described from Japan (Ichimura 1918, Imazeki & Hongo 1957, Imazeki et al. 1988; Romagnesi 1989, Guez 1990), also occurs in South Korea (Lee & Hong 1985). It was reported as growing on both angiosperm (*Phyllostachys bambusoides*, *Acer palmatum*, *Zelkova serrata*) and gymnosperm (*Cryptomeria japonica*) litter.

Phylogeny and specific delimitation

In our combined ITS-LSU phylogenetic tree (FIG. 1) *Clitocybe amoenolens* and its sister *C. acromelalga* are not closely related to *C. nebularis* (Batsch) P. Kumm., the type of the genus *Clitocybe* (Redhead et al. 2002), nor to other *Clitocybe* species or allied taxa. As these two species represent a new phyletic line of clitocyboid fungi, it seems most appropriate to transfer them to the new genus *Paralepistopsis*.

Based on its habit, coloured lamellae and small spores, Bon (1997) and Moreau et al. (2001) placed *C. amoenolens* traditionally in subg. *Clitocybe* sect. *Gilvaoideae* Harmaja, where it occupies an isolated position. Contu et al. (1999), focusing on hymenial features (basidia longer than 30 µm), placed it in subgen. *Hygroclitocybe* Bon sect. *Clavipedes* Harmaja, a subgenus shown in recent molecular analyses (Redhead et al. 2002, Vizzini et al. 2011) to be artificial and heterogeneous.

The traditionally defined *Lepista* (Fr.) W.G. Sm. —clitocyboid fungi with a pinkish yellow spore print, usually separable lamellae, and inamyloid cyanophilous ornamented [verruculose to spiny] basidiospores (Singer 1986, Bon 1997, Consiglio & Contu 2003)— is a polyphyletic genus (FIG. 1). The species of *Lepista* subg. *Paralepista* (= *Lepista* sect. *Gilva*), which combine very crowded decurrent lamellae with subglobose to largely ellipsoidal spores, are not closely related either to *Lepista* s.s. or to other taxa in the tricholomatoid clade. Consequently we accept this lineage as a distinct genus and propose *Paralepista* for *Lepista flaccida*, *L. gilva* and allies. Following Bigelow (1985), Bon (1991), and Raitelhuber (2004), we list below all the taxa accepted in *Paralepista*:

***Paralepista* Raitelhuber, Gattung *Clitocybe* 1: 17 (1981).**

TYPE SPECIES — *Agaricus inversus* Scop.

= *Lepista* subg. *Paralepista* (Raitelhuber) Bon, Doc. Mycol. 26(102): 18 (1996).

= *Clitocybe* sect. *Eulepistae* Singer, Ann. Mycol. 41: 40 (1943).

= *Lepista* sect. *Gilva* Harmaja, Karstenia 18: 53 (1978).

"*Lepista* sect. *Eulepista*" Konrad & Maubl., Icon. Select. Fung. 6(10): 350 (1936), nom. inval.

"*Lepista* sect. *Inversae*" Singer & Cléménçon, Nova Hedwigia
23: 310 (1973 ["1972"]), nom. inval.

***Paralepista abdita* (Dörfelt) Vizzini, comb. nov.**

MYCOBANK MB 564343

= *Lepista abdita* Dörfelt, Boletus 1(2): 37 (1997).

***Paralepista ameliae* (Arcang.) Vizzini, comb. nov.**

MYCOBANK MB 564344

= *Clitocybe spinulosa* var. *ameliae* Arcang., Nuovo Giorn. Bot. Ital. 21: 434 (1889).

***Paralepista biformis* (Peck) Vizzini, comb. nov.**

MYCOBANK MB 564345

= *Clitocybe biformis* Peck, Bull. N.Y. St. Mus. 150: 25 (1911).

***Paralepista concentrica* Raithelh., Metrodiana 23: 122 (1996).**

***Paralepista femoralis* (H.E. Bigelow) Vizzini, comb. nov.**

MYCOBANK MB 564346

= *Clitocybe femoralis* H.E. Bigelow, Sydowia 36: 14 (1983).

***Paralepista flaccida* (Sowerby) Vizzini, comb. nov.**

MYCOBANK MB 564347

= *Agaricus flaccidus* Sowerby, Col. Fig. Engl. Fungi 2: pl. 185 (1799).

***Paralepista flaccida* var. *fibrillosa* (Malençon) Vizzini, comb. nov.**

MYCOBANK MB 564348

= *Clitocybe flaccida* var. *fibrillosa* Malençon, in Malençon & Bertault,
Flore des champignons superieurs du Maroc 2 - Trav. Inst.
Sci. Chérifien, Sér. Bot. Biol. Vég. 33: 157 (1975).

***Paralepista gilva* (Pers.) Vizzini, comb. nov.**

MYCOBANK MB 564349

= *Agaricus gilvus* Pers., Syn. Meth. Fung.: 448 (1801).

"*Paralepista gilva*" Raithelh., Metrodiana 23: 117 (1996), nom. inval.

***Paralepista inversa* (Scop.) Raithelh., Gattung *Clitocybe* 1: 17 (1981).**

= *Agaricus inversus* Scop., Fl. Carniol., Ed. 2, 2: 445 (1772).

***Paralepista lentiginosa* (Fr.) Vizzini, comb. nov.**

MYCOBANK MB 564350

= *Agaricus lentiginosus* Fr., Epicr. Syst. Mycol.: 69 (1838).

***Paralepista maculosa* (Sacc.) Vizzini, comb. nov.**

MYCOBANK MB 564352

= *Agaricus maculosus* Peck, Bull. Buffalo Soc. Nat. Sci. 1:
45 (1873), nom. illegit., non Pers. (1801).

= *Clitocybe maculosa* Sacc., Syll. Fung. 5: 183 (1887).

***Paralepista pseudoparilis* (Enderle & Contu) Vizzini, comb. nov.**

MYCOBANK MB 564353

= *Lepista pseudoparilis* Enderle & Contu, Beitr. Kenn. Pilze Mittel. 13: 12 (2000).

***Paralepista repanda* (Raithelh.) Raithelh., Metrodiana 23: 121 (1996).**

= *Lepista repanda* Raithelh., Metrodiana 14: 21 (1986 ["1985"]).

***Paralepista shafferi* (H.E. Bigelow) Vizzini, comb. nov.**

MYCOBANK MB 564354

= *Clitocybe shafferi* H.E. Bigelow, Beih. Nova Hedwigia 81: 339 (1985).

***Paralepista splendens* (Pers.) Vizzini, comb. nov.**

MYCOBANK MB 564355

= *Agaricus splendens* Pers., Syn. Meth. Fung.: 452 (1801).

Discussion

Paralepistopsis species are characterized by a habit (decurrent and crowded lamellae) and colours (ochre-orange tinges) reminiscent of *Paralepista* or *Infundibulicybe* Harmaja, a whitish to cream spore print, smooth cyanophilic spores often arranged in tetrads in dried specimens and rarely exceeding 5(–6) µm in length. *Paralepista* differs in having strongly ornamented spores (Raithelhuber 1995, 2004); *Infundibulicybe* is distinguished by smooth lacrymoid spores with confluent bases and cyanophobic spore walls (Harmaja 2003).

Paralepistopsis clusters with *Cleistocybe* Ammirati et al. and *Catathelasma* in the /*catathelasma* clade. Because of the low resolution and lack of BPP and MLB support within the tree, a more precise, accurate position for the new genus could not be suggested. The presence of decurrent lamellae, confluent pileus and stipe, pale reddish brown colouration, and growth on soil are characters shared by *Paralepistopsis*, *Cleistocybe*, and *Catathelasma*. *Cleistocybe* and *Catathelasma* are distinguished from *Paralepistopsis* mainly by a partial veil, divergent to interwoven hymenophoral trama, and larger cyanophobic spores (Ammirati et al. 2007, Vizzini 2009); in addition *Catathelasma* spores are amyloid (Singer 1986).

Paralepistopsis amoenolens is delimited by a unique combination of macro-/micromorphological and chemical features, such as i) a strong aromatic, floral odour reminiscent of *Tricholoma caligatum*, *Inocybe corydalina*, *Lepista irina*, and *Entoloma ameides* caused by volatile metabolites identified by Fons et al. (2006) as methyl-(E)-cinnamate (also a key odorant of *T. caligatum*), methylbenzoate, (E)-nerolidol, and methylantranilate; ii) lamellae easily separating from the pileus context; iii) a cream coloured spore print; iv) smooth cyanophilic spores often arranged in tetrads; v) basidia reaching 35–40 µm; vi) pileipellis hyphae with short diverticula; vii) abundant thromboplerous hyphae; viii) and the presence of the toxic metabolite, acromelic acid A, a powerful neurotoxic

amino acid responsible for erythromelalgic poisoning and structurally homologous with kainic acid (a strong agonist of non-*N*-methyl-*D*-aspartate glutamate receptor subtypes) and domoic acid (Bessard et al. 2004).

Paralepistopsis acromelalga differs from *P. amoenolens* morphologically in a darker pileus and stipe, a pileus that soon becomes depressed, more crowded lamellae, a different odour, thromboplerous hyphae occurring only rarely, smaller spores (Ichimura 1918, Romagnesi 1989, Guez 1990, Miyauchi 1998, Moreau et al. 2001), and a more complex metabolite pattern (presence of acromelic acids A–E with 19 other toxins; Konno et al. 1983, 1988; Fushiya et al. 1990, 1992; Saviuc & Danel 2006). Additionally, our analyses show only a 91% pairwise ITS sequence identity between *P. acromelalga* and *P. amoenolens*. Singer (1986) transferred *P. acromelalga* to the heterogoneous genus *Neoclitocybe* Singer based on the presence of rare diverticulate hyphae in the pileipellis.

Based on their small spores and the *Paralepista*-like habit, *C. gilvaoides* Kauffman and *C. gracilis* (H.E. Bigelow & A.H. Sm.) Harmaja (sect. *Gilvaoidae*) from the coniferous forests of North America and Scandinavia (Bigelow 1985, Harmaja 1969) may also belong to *Paralepistopsis*, but more recently collected specimens are needed to perform molecular and biochemical analyses.

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Three new species of *Septobasidium* (Septobasidiaceae) from Hainan Province in ChinaSUZHEN CHEN^{1,2} & LIN GUO^{1*}¹State Key Laboratory of Mycology, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China²Ocean University of China, Qingdao 266003, China* CORRESPONDENCE TO: guol@im.ac.cn

ABSTRACT —Three new *Septobasidium* species are described: *S. capparis* on *Capparis membranifolia* associated with *Andaspis* sp., *S. reevesiae* on *Reevesia longipetiolata* associated with *Lepidosaphes* sp., and *S. dacrydii* on *Dacrydium pierrei* associated with *Pinnaspis* sp. They were collected from Hainan Province, China.

KEY WORDS —*Pucciniomycetes*, *Septobasidiales*, taxonomy

To date, nine new species and three new Chinese records of *Septobasidium* have been discovered from Hainan Province (Chen & Guo 2011b,c,d, 2012, Lu & Guo 2009a, 2010b,c). An additional three new species of *Septobasidium* are reported as follows:

***Septobasidium capparis* S.Z. Chen & L. Guo, sp. nov.**

FIGS 1–6

FUNGAL NAME FN570003

Differs from *Septobasidium euryae-groffii* by its thicker hymenium and larger basidia.

TYPE: China, Hainan Province, Changjiang, Baomeiling, alt. 250 m, on *Capparis membranifolia* Kurz (*Capparaceae*), associated with *Andaspis* sp. (*Diaspididae*), 12.IV.2011, L. Guo 11599 (HMAS 263233, *holotype*).

ETYMOLOGY: The epithet refers to the substrate plant genus, *Capparis*.

Basidiomata on branches, resupinate, 10–24 cm long, 1.5–4 cm wide, pale cinnamon-brown or brown; margin determinate, surface smooth, cracked at maturity. In section 1500–2000 µm thick, composed of 3–8 layers. Subiculum brown, 40–70 µm thick. Pillars brown, 80–100 µm high, 70–130 µm wide, branched outwards to form a hyphal layer, 500–550 µm high. From hymenial layer the fungal hyphae renew growth successively to form hyphal layers and

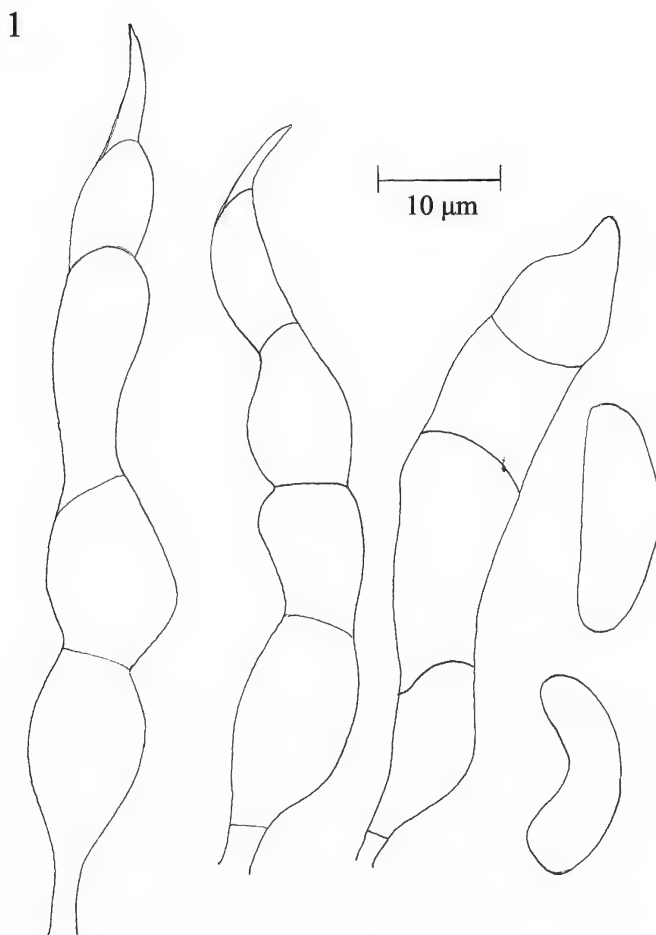
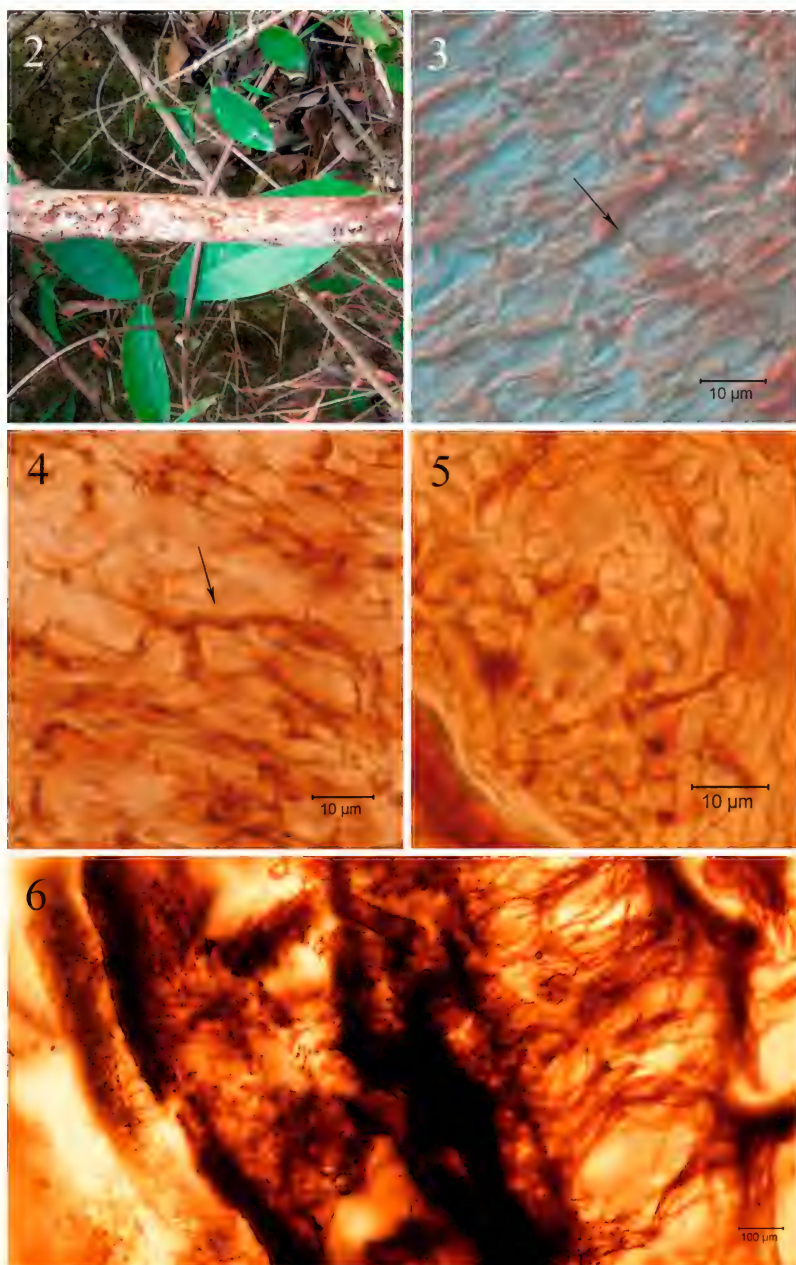


FIG. 1. *Septobasidium capparis* (HMAS 263233, holotype). Basidia and basidiospores.

hymenia. Hymenium brown, 100–170 μm thick. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, straight or slightly curved, 4-celled, $45\text{--}56 \times 8\text{--}12 \mu\text{m}$, hyaline or brown. Sterigmata conical, $6\text{--}8 \times 3\text{--}5 \mu\text{m}$, hyaline or brown. Basidiospores reniform, $16\text{--}20 \times 4.5\text{--}8 \mu\text{m}$, hyaline. Haustoria consisting of irregularly coiled hyphae.

COMMENTS: *Septobasidium capparis* is similar to *S. euryae-groffii* C.X. Lu & L. Guo, which differs by its thinner hymenium (70–110 μm) and smaller basidia ($20\text{--}45 \times 5\text{--}8 \mu\text{m}$; Lu & Guo 2010a).



FIGS. 2–6 *Septobasidium capparidis* (HMAS 263233, holotype). 2. Basidioma on branch. 3–4. Basidia (arrows). 5. Haustoria. 6. Section of basidioma.

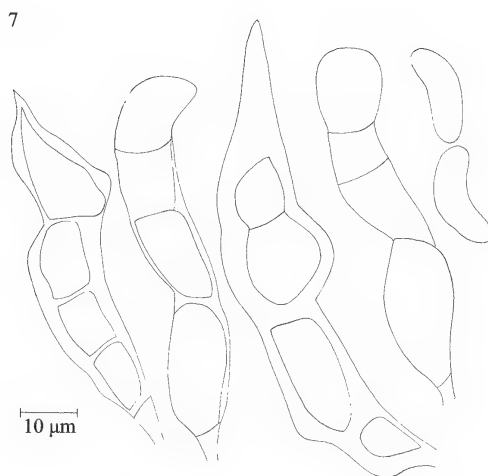


FIG. 7. *Septobasidium reevesiae* (HMAS 263427, holotype). Basidia and basidiospores.

***Septobasidium reevesiae* S.Z. Chen & L. Guo, sp. nov.**

FIGS 7–12

FUNGAL NAME FN570004

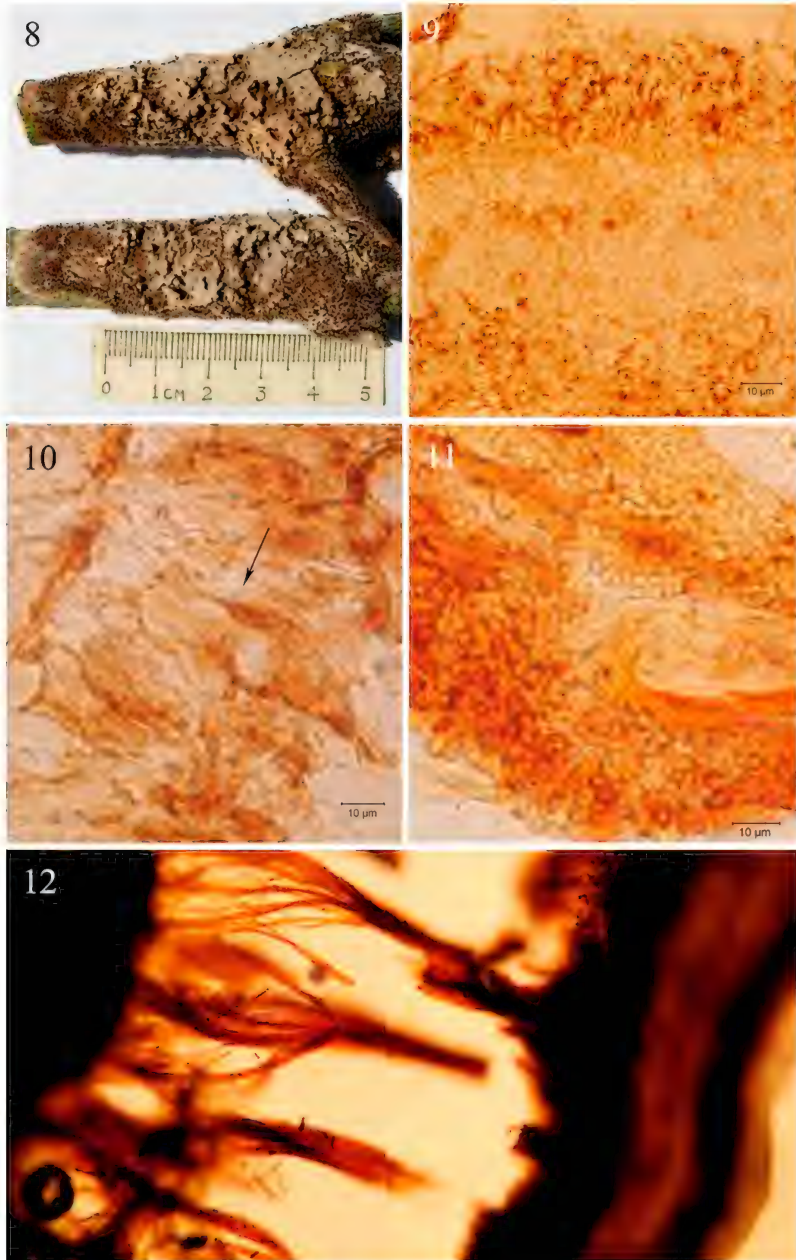
Differs from *Septobasidium henningsii* by its upright pillars and thicker stratified hymenial layer.

TYPE: China, Hainan Province, Bawangling Natural Reserve, alt. 1030 m, on *Reevesia longipetiolata* Merr. & Chun (Sterculiaceae), associated with *Lepidosaphes* sp. (Diaspididae), 25.XI.2010, Y.F. Zhu & F. He 518 (HMAS 263427, holotype).

ETYMOLOGY: The epithet refers to the substrate plant genus, *Reevesia*.

Basidiomata on branches, resupinate, 7.5–8.5 cm long, 3–6 cm wide, cinnamon-brown; margin white, determinate, surface smooth, at first frequently forming orbiculate or discoideous patches, confluent in the old stage. The dense pillars are barely visible, especially near the margin. In section 1650–2200 μm thick. Subiculum brown, 30–50 μm thick. Pillars fasciculate, brown, 700–1170 μm high, 40–100 μm wide. Hymenial layer hyaline or brown, 730–1000 μm thick, often forming 2–4 strata, with a brown, 2–7 μm thick horizontal layer between the hymenia, and closely packed parallel upright hyphae. Basidia arising directly from the hyphae without a probasidial cell, cylindrical, straight or slightly curved, 4-celled, 37–55 × 8–13(–18) μm, hyaline or brown; wall 1–2(–3) μm thick. Sterigmata conical, 9–11(–23) × 3–4.5 μm. Basidiospores reniform, 15–16 × 5–6 μm. Haustoria consisting of irregularly coiled hyphae.

COMMENTS: *Septobasidium reevesiae* is similar to *S. henningsii* Pat., which differs by its thinner hymenial layer (60–400 μm high) and characteristic slanting, entangled, anastomosing pillars (Couch 1938).



FIGS. 8–12. *Septobasidium reevesiae* (HMAS 263427, holotype). 8. Basidiomata on branches. 9. Stratified hymenial layer. 10. Basidia (arrow). 11. Haustoria. 12. Section of basidioma.

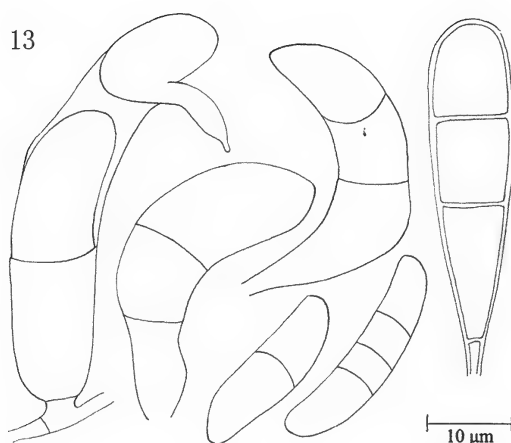


FIG. 13. *Septobasidium dacrydii* (HMAS 263232, holotype). Basidia and basidiospores.

***Septobasidium dacrydii* S.Z. Chen & L. Guo, sp. nov.**

FIGS 13–18

FUNGAL NAME FN570005

Differs from *Septobasidium apiculatum* by its thicker section with pillars.

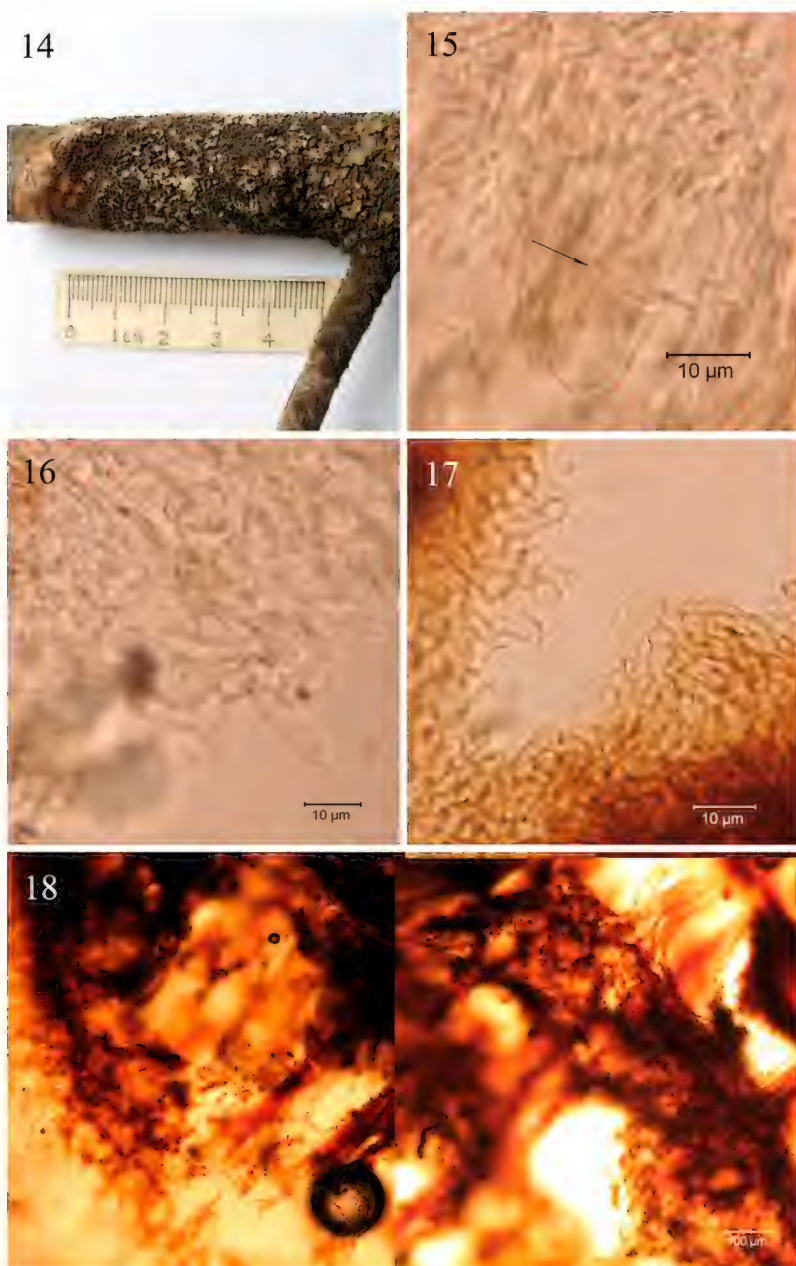
TYPE: China, Hainan Province, Bawangling Natural Reserve, alt. 1050 m, on *Dacrydium pierrei* Hickel (*Podocarpaceae*), associated with *Pinna sp.* (*Diaspididae*), 24.XI.2010, Y.F. Zhu & F. He 529 (HMAS 263232, **holotype**).

ETYMOLOGY: The epithet refers to the substrate plant genus, *Dacrydium*.

Basidiomata on branches, resupinate, 35 cm long, 1–6 cm wide, cinnamon-brown or dark brown; margin determinate, surface smooth, peeled off in the old stage, dark brown pillars and hyphal layers emerging. In section 1450–2000 μm thick. Subiculum brown, 45–70 μm thick. From the subiculum forming pillars or hyphal layers. Pillars brown, higher or shorter, 850–1200 μm high, 35–110 μm wide, or 100–200 μm high, 40–80 μm wide. Hyphal layer, 700–800 μm high. Pillars and hyphal layers successively formed from the hyphal layer. Hymenium hyaline, 85–140 μm thick. Basidia arising directly from the hyphae without a probasidial cell, cylindrical or clavate, straight or slightly curved, 3-celled, 30–40 $\times$ 9–10 μm , hyaline. Sterigmata conical, 10–15 $\times$ 4–5 μm , hyaline. Basidiospores cylindrical or fusiform, 21–26 $\times$ 5–6.5 μm , hyaline. Haustoria consisting of irregularly coiled hyphae.

COMMENTS: *Septobasidium dacrydii* is similar to *S. apiculatum* Couch ex L.D. Gómez & Henk, which differs by its thinner section (250–550 μm thick) and absence of pillars (Couch 1938).

Including the three new species reported in this paper, 46 *Septobasidium* species have now been reported in China (Sawada 1933, Couch 1938, Teng 1963, Tai



FIGS. 14–18. *Septobasidium dacrydii* (HMAS 263232, holotype). 14. Basidioma on branch. 15. Basidia (arrow). 16. A basidiospore. 17. Haustoria. 18. Section of basidioma.

1979, Kirschner & Chen 2007, Lu & Guo 2009a,b,c, 2010a,b,c, 2011, Lu et al. 2010, Chen & Guo 2011a,b,c,d, 2012).

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Characterization of the causal agent of poplar anthracnose occurring in the Beijing regionZHENG LI¹, YING MEI LIANG² & CHENG MING TIAN^{1*}

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ABSTRACT — Twenty fungal isolates derived from infected poplar leaves collected in four Beijing regional districts during 2009 and 2010 were examined for morphological and cultural characteristics. Multi-gene phylogenetic sequence analyses of the rDNA ITS (ITS1–5.8S–ITS2) region and the β -tubulin2, partial actin (ACT), glyceraldehyde-3-phosphate dehydrogenase (GPDH), and glutamine synthetase (GS) genes were performed for these isolates. The morphological and cultural evaluations and DNA sequence analyses demonstrated that 16 isolates represented *Colletotrichum gloeosporioides* while the remaining four isolates, all from Shi Jingshan district, represent a new species, *Colletotrichum populi*, which is described and illustrated.

KEY WORDS — disease, multi-locus phylogeny, taxonomy

Introduction

The genus *Colletotrichum* causes anthracnose disease on a wide range of plants worldwide. The main *Colletotrichum* species causing anthracnose of forest trees include *C. gloeosporioides*, *C. acutatum*, and *C. crassipes*, which may cause infections of leaves and fruits of plants such as poplar, China fir, and paulownia, often leading to premature defoliation (Xu et al. 2004). Of these, *C. gloeosporioides* is the most common, affecting a wide range of tree hosts. The taxonomy of *C. gloeosporioides* is extremely complex because of its heterogeneous nature and instability in cultural characteristics. For example, more than 600 *C. gloeosporioides* synonyms have been identified (von Arx 1957). Different experimental conditions and measurement errors may have a marked influence over the recorded sizes of conidia. Identification results obtained through traditional morphological examination are thought to be unreliable (Cai et al. 2009) when compared to molecular methods, and several new species, such as *C. asianum* and *C. cordylinicola* (Prihastuti et al. 2009,

TABLE 1. Isolates of *Colletotrichum* used in study.

ISOLATE NO.	HOST	ORIGIN	SAMPLING DATE
1C5-7	<i>Populus ×beijingensis</i>	Chang ping	2010.08
C1-5-1	<i>Populus ×beijingensis</i>	Chang ping	2010.08
2C5-8	<i>Populus ×beijingensis</i>	Chang ping	2010.08
2C5-9	<i>Populus ×beijingensis</i>	Chang ping	2010.08
2C5-14	<i>Populus ×beijingensis</i>	Chang ping	2010.08
3C5-3	<i>Populus ×beijingensis</i>	Chang ping	2010.08
4C5-2	<i>Populus ×beijingensis</i>	Chang ping	2010.08
C2-5	<i>Populus ×beijingensis</i>	Chang ping	2010.07
Y3-4	<i>Populus cathayana</i>	Yan qing	2010.08
Y3-6	<i>Populus cathayana</i>	Yan qing	2010.08
Yp1-7	<i>Populus cathayana</i>	Yan qing	2010.08
Bh7-2	<i>Populus ×beijingensis</i>	Yan qing	2010.09
Bh9-1	<i>Populus cathayana</i>	Yan qing	2010.09
Bh12-2	<i>Populus ×beijingensis</i>	Yan qing	2010.09
Dy10-2	<i>Populus nigra</i>	Yan qing	2010.09
HMBFU191	<i>Populus nigra</i> var. <i>italica</i>	Shi Jingshan	2009.10
HMBFU163	<i>Populus nigra</i> var. <i>italica</i>	Shi Jingshan	2009.10
HMBFU173	<i>Populus nigra</i> var. <i>italica</i>	Shi Jingshan	2009.10
HMBFU141	<i>Populus nigra</i> var. <i>italica</i>	Shi Jingshan	2009.10
M1-6	<i>Populus nigra</i>	Mi yun	2010.08

Phoulivong et al, 2010a), have recently been separated from *C. gloeosporioides* based on the results from molecular analysis.

Colletotrichum gloeosporioides (teleomorph *Glomerella cingulata*) has been reported as the main pathogen of poplar anthracnose in China and North America (He et al. 1990, Newcombe 2000). *Colletotrichum graminicola*, which usually infects monocotyledonous plants such as cereals or grasses, may also infect poplar in India (Dipak et al. 1999). Most studies that are based on morphology may be contradicted by molecular data (Phoulivong et al. 2010b).

The present study was carried out to determine the *Colletotrichum* species associated with poplar anthracnose in Beijing area through morphological examination and molecular methods.

Materials & methods

Isolation of *Colletotrichum* species

Twenty *Colletotrichum* isolates were obtained from lesions on infected leaves of different poplar species collected in four districts in Beijing: Chang ping, Yan qing, Mi yun, and Shi Jingshan (TABLE 1). These isolates are deposited at the Mycological Herbarium of Beijing Forestry University (BJFC).

Three 5×5 mm samples cut from the interface of healthy and diseased tissue were surface sterilized by dipping in 70% ethanol for 1 minute, then in 1% sodium hypochlorite for 3–4 minutes and washing in three changes of sterile water. The leaf segments were then placed onto the surface of potato dextrose agar (PDA) and incubated

at room temperature (28°C). The growing edges of fungal hyphae developing from the leaf segments were transferred onto a new PDA plate after 1–2 days. Each isolate was purified through single spore isolation and was maintained on a new PDA plate at 28°C for further study.

Morphological studies

Mycelial discs (5 mm diam) derived from the edge of a 7-day old culture were transferred onto PDA and incubated at 28°C in the dark. Each colony radius was measured daily after the 2nd day for 4 days to determine the growth rate by averaging the daily growth (cm per day) of the colonies. Colony characters were also recorded and described. After 7 days a spore suspension (5×10^5 conidia/ml) was made from each culture and the size and shape of 30 conidia were measured by light microscopy at a magnification of 40x. A drop of the spore suspension was placed on a slide, which was placed in a Petri dish containing water-soaked filter paper to keep moist. After 24 hours (at 28°C) conidial germination was observed, and the size and shape of 20 appressoria were measured.

Statistical data (morphology and growth rate) were analyzed using SPSS software version 11.5.0 (SPSS Inc., USA).

DNA extraction and PCR amplification

Mycelium was scraped from the surface of 7-day old cultures of each isolate and dried using sterilized filter paper. Genomic DNA was extracted using the modified CTAB method. Extracted DNA was electrophoresed on 1% agarose gel to check genomic DNA quality.

Using polymerase chain reaction (PCR), DNA was amplified from the complete rDNA-ITS (ITS) region and the β -tubulin (TUB2), partial actin (ACT), glutamine synthetase (GS), and glyceraldehyde-3-phosphate dehydrogenase (GPDH) genes of each *Colletotrichum* isolate. The primer pairs for the amplified genes are ITS1+ITS4 (White et al. 1990), Bt2a+Bt2b (Glass et al. 1995), ACTF+ACTR (Carbone & Kohn 1999), GSF1+GSR1 (Guerber et al. 2003), GDF1+GDR1 (Peres et al. 2008) respectively. Amplification was performed in 25 μ L volume with 1 μ L (20 ng) DNA template, 2 μ L dNTP (2.5 mmol/L), 2.5 μ L 10 $\times$ PCR buffer, 0.5 μ L of each primer (10 μ Lmol/L), 0.15 μ L Taq DNA polymerase and 18.35 μ L ddH₂O. The PCR profile consisted of denaturation at 95°C for 3 min, followed by 34 cycles at 95°C for 1 min, 55°C for 30 s, 72°C for 1 min and a final cycle at 72°C for 10 min. PCR products were electrophoresed on 1% agarose gel and DNA sequencing was carried out by the Shanghai Invitrogen Biological Technology Co. Errors were adjusted manually with BioEdit software version 7.0.5.3 (© Tom Hall). All isolate and DNA sequence accession (DDBJ, EMBL, GenBank) numbers are listed in TABLE 2.

Phylogenetic analysis

The sequences of *Colletotrichum* isolates were aligned using Clustal X (1.81). Alignments were manually adjusted to allow maximum alignment and maximum sequence similarity, and gaps were treated as missing data.

For parsimony analysis, PAUP version 4.0b10 was used and heuristic search was performed with 1000 repeats of random addition sequences in Stepwise-Addition Option and TBR swapping algorithm in Branch Swapping Option. Confidence limits

TABLE 2. *Colletotrichum* isolates used in this study.*

SPECIES	ISOLATE No.	HOST	LOCATION	DATABASE ACCESSION No. [^]					
				ITS	β-TUBULIN	ACT	GPDH	GS	
<i>C. asiaticum</i>	BPD-14	Coffee	Thailand	FJ972612	FJ907439	FJ907424	FJ972576	FJ972595	
<i>C. acutatum</i>	CBS294.67	Papaya	Australia	FJ972610	FJ907444	FJ907429	FJ972581	FJ972590	
<i>C. boninense</i>	C05016	Ginseng	Korea	GU935883	GU935903	GU935804	GU935863	GU935823	
<i>C. cordylinicola</i>	LC0856	<i>Cordyline fruticosa</i>	Thailand	HM470246	HM470249	HM470234	HM470240	HM470243	
<i>C. fruticola</i>	BPD-112	Coffee	Thailand	FJ972611	FJ907440	FJ907425	FJ972577	FJ972594	
<i>C. gloeosporioides</i>	CBS953.97	Citrus	Italy	FJ972609	FJ907445	FJ907430	FJ972582	FJ972589	
<i>C. gloeosporioides</i>	BH9-1	Poplar	China	AB632351	JN862897	JN184702	JN211078	JN211087	
<i>C. gloeosporioides</i>	4C5-2	Poplar	China	AB632352	JN862882	JN184700	JN211065	JN211097	
<i>C. gloeosporioides</i>	BH7-2	Poplar	China	AB632353	JN862883	JN184701	JN211080	JN211091	
<i>C. gloeosporioides</i>	Yp1-7	Poplar	China	AB632354	JN862884	JN184698	JN211075	JN211095	
<i>C. gloeosporioides</i>	1C5-7	Poplar	China	AB632355	JN862885	JN184699	JN211074	JN211086	
<i>C. gloeosporioides</i>	2C5-9	Poplar	China	AB632356	JN862886	JN184697	JN211073	JN211090	
<i>C. gloeosporioides</i>	Dy10-2	Poplar	China	AB632357	JN862896	JN184692	JN211077	JN211092	
<i>C. gloeosporioides</i>	3C5-3	Poplar	China	AB632358	JN862887	JN184705	JN211072	JN211098	
<i>C. gloeosporioides</i>	Y3-6	Poplar	China	AB632359	JN862895	JN184694	JN211071	JN211093	
<i>C. gloeosporioides</i>	C2-5	Poplar	China	AB632360	JN862894	JN184695	JN211070	JN211088	
<i>C. gloeosporioides</i>	2C5-14	Poplar	China	AB632361	JN862893	JN184696	JN211076	JN211099	
<i>C. gloeosporioides</i>	Y3-4	Poplar	China	AB632362	JN862892	JN184689	JN211069	JN211085	
<i>C. gloeosporioides</i>	M1-6	Poplar	China	AB632363	JN862891	JN184690	JN211066	JN211094	
<i>C. gloeosporioides</i>	2C5-8	Poplar	China	AB632364	JN862890	JN184691	JN211068	JN211096	
<i>C. gloeosporioides</i>	BH12-2	Poplar	China	AB632365	JN862889	JN184687	JN211079	JN211100	
<i>C. gloeosporioides</i>	C1-5-1	Poplar	China	AB632366	JN862888	JN184688	JN211067	JN211089	
<i>C. horii</i>	TSG002	<i>Mangifera indica</i>	China	AY791890	GU13380	GU13337	GQ329680	GU133381	
<i>C. hymenocallidis</i>	CSN3	<i>Hymenocallis</i>	China	GQ485601	GQ849439	GQ856776	GQ856759	—	
<i>C. jasmmini-sambac</i>	LTIX-01	<i>Jasminum sambac</i>	China	HM131511	HM153768	HM131507	HM131497	HM131502	
<i>C. kahawae</i>	IM319418	Coffee	Kenya	FJ972608	FJ907446	FJ907432	FJ972583	FJ972588	
<i>C. populi</i>	HMBFU191	Poplar	China	AB632347	JN862898	JN184704	JN211081	JN211101	
<i>C. populi</i>	HMBFU163	Poplar	China	AB632348	JN862899	JN184706	JN211082	JN211102	
<i>C. populi</i>	HMBFU173	Poplar	China	AB632349	JN862900	JN184707	JN211084	JN211104	
<i>C. populi</i>	HMBFU141	Poplar	China	AB632350	JN862901	JN184708	JN211083	JN211103	
<i>C. siamense</i>	BML-115	Coffee	Thailand	FJ972614	FJ907437	FJ907422	FJ972574	FJ972597	

* Isolates and sequences generated by this research are indicated in bold. [^] Accession numbers are all available in DDBJ (DNA Database of Japan), EMBL, and GenBank.

for the branches based on parsimony criteria were estimated by bootstrap analysis of 1000 replicates. Phylogenetic trees were constructed from distance matrix values by the maximum parsimony (MP) method. Describing trees was based on tree length, consistency index (CI), retention index (RI), rescaled consistency index (RC) and homoplasy index (HI). Constructed trees were viewed by TreeView software. The GenBank accession numbers used as references in this study are listed in TABLE 2.

Results

Morphological and cultural characterization

The isolates could be divided into two groups (TABLE 3) based on differences in cultural characteristics and appressorial morphology. Group 1 consisted of 16 isolates with colonies varying from white or grey to dark brown and turning dark grey or olive-green with time; aerial mycelium white or grey (PLATE 1A–B). These cultures produced abundant orange conidial masses around the inoculation point. The conidia were cylindrical, obtuse at both ends or slightly tapered at one end, possessing 2–3 vacuoles and measuring $9.8\text{--}22.0 \times 2.9\text{--}6.9 \mu\text{m}$ (PLATE 1C). Appressoria were generally round or ovate and $4.9\text{--}15.9 \times 4.4\text{--}9.8 \mu\text{m}$ (PLATE 1D).

TABLE 3. Morphological and cultural characteristics of 20 *Colletotrichum* isolates. *

	<i>C. gloeosporioides</i> (GROUP 1)	<i>C. populi</i> (GROUP 2)
ISOLATES TESTED	16	4
CONIDIA		
SHAPE	Cylindrical	Cylindrical
LENGTH (μm)	15.8 ± 1.9 (9.8–22.0)	15.1 ± 2.0 (9.8–17.7)
WIDTH (μm)	4.9 ± 0.4 (2.9–6.9)	5.2 ± 0.9 (3.0–7.3)
APPRESSORIA		
SHAPE	Circular or ovate	Irregular, edge lobed
LENGTH (μm)	9.4 ± 2.1 (4.9–15.9)	9.4 ± 1.6 (7.3–12.2)
WIDTH (μm)	5.9 ± 1.0 (4.4–9.8)	6.2 ± 1.2 (4.9–9.3)
COLONY	Light, dark grey or brown; aerial mycelium grey with orange conidial masses	Aerial mycelium cottony, white, with indistinct conidial masses
DAILY GROWTH (cm/d)	0.72 ± 0.08 (0.56–0.88)	0.73 ± 0.05 (0.62–0.80)

* Measurements are presented as mean $\pm$ sd (range).

Group 2 consisted of four isolates collected from Shi Jingshan district. The colonies were white to light grey in color and covered with white cottony aerial mycelia, with indistinct conidia masses on the surface of colonies. Conidia were similar to those of Group 1 in shape (cylindrical) and size ($9.8\text{--}17.7 \times 3.0\text{--}7.3 \mu\text{m}$), but had 1–2 vacuoles. Appressoria were irregular in shape with lobed edges and measured $7.3\text{--}12.2 \times 4.9\text{--}9.3 \mu\text{m}$.

There were no significant differences in growth rate between the two groups, both showing an average daily growth of ca. 0.72 cm/d.

Multi-locus phylogeny

The sequence information obtained from five genes and the phylogenetic tree information based on each gene sequence are shown in TABLE 4. Of the five genes, GS and GPDH had the highest rates of parsimony informative characters (PIC), GS comprising 439 (40.1%) PIC out of a total 1095 characters and GPDH 114 (38.6%) PIC out of 295 characters.

The phylogenetic tree was constructed on the basis of combined data of the five genes (PLATE 2). The best tree contained 2593 characters with NPIC=681, CI=0.865, RI=0.853, HI=0.135, RC=0.738 and Length=1712, showing that the two morphologically distinct groups were placed into two separate branches. Group 1 clustered with *C. gloeosporioides* with a bootstrap value of 100%. Group 2 was distinctive from *C. fructicola* (bootstrap value = 79%) and other members in ‘*gloeosporioides* complex’ (bootstrap values > 90%), suggesting the probability of a new species.

TABLE 4. Gene regions amplified.

DNA REGION	SEQUENCE LENGTHS (BP)	PHYLOGENETIC TREE		
		CHARACTERS	NPIC	NPIC/CHARACTERS (%)
ITS	534–537	563	45	8.0
TUB2	440–441	457	84	18.4
ACT	241	299	60	20.1
GPDH	221–230	295	114	38.6
GS	981–988	1095	439	40.1

Taxonomy

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. PLATE 1

Lesions on leaves of poplar as black spots distributed randomly or in circles with a dark brown border, orange conidial masses sometimes observed. Conidia cylindrical, 9.8–22.0 × 2.9–6.9 µm, obtuse at ends, hyaline, smooth. Appressoria ovate or circular, 4.9–15.9 × 4.4–9.8 µm. Colonies on PDA with white, grey, dark grey, or olive grey mycelium and orange sporulation. Reverse brown or olive-green with dark spots.

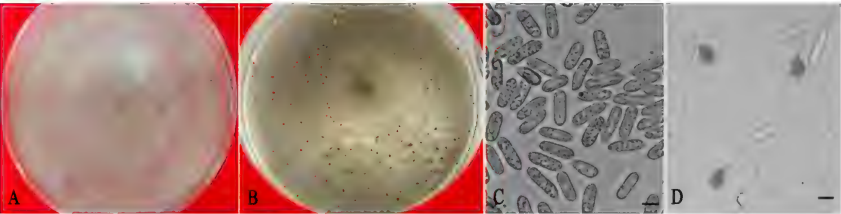


PLATE 1. *Colletotrichum gloeosporioides* isolates (Group 1): cultural and morphological characters. A: Colony of isolate 2C5-14; B: Colony of isolate BH7-2; C: Conidia of isolate M1-6; D: Clavate or circular appressoria of isolate C1-5-1. Bars = 10 µm.

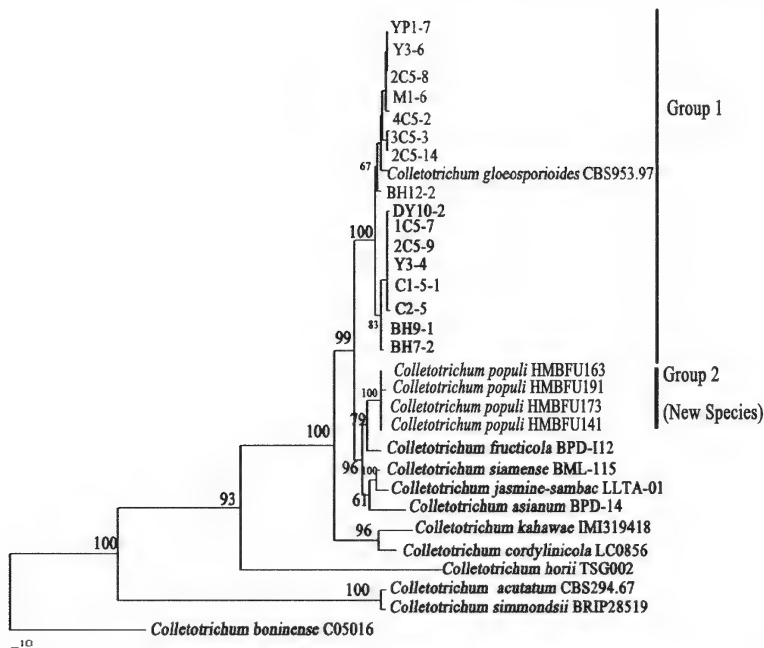


PLATE 2. Phylogenetic tree based on the combination of sequences of ITS, TUB2, ACT, GPDH, and GS genes (MP.)

Colletotrichum populi C.M. Tian & Zheng Li, sp. nov.

MYCOBANK 564436

Lesions with black spots in circle and a light brown border, sometimes sunken. Differs from *Colletotrichum fructicola* by grey-white PDA colonies (reverse grey white or pale yellow) and from *C. gloeosporioides* by longer and irregularly lobed appressoria formed from conidia.

TYPE: China. Beijing, Shi Jingshan District, from leaves of *Populus nigra* var. *italica*, 25th October 2009, coll. C.M. Tian, HMBFU191 (Holotype BJFC, lyophilized PDA culture).

ETYMOLOGY: *populi*, referring to the host species.

On leaves of poplar producing circular black spots with a dark brown border. Colonies on PDA at first white, becoming grey-white, reverse grey white or pale yellow, in 5 days at 28°C, daily growth 0.62–0.80 cm/day (mean = 0.73 ± 0.05 , n = 5). Aerial mycelium cottony, white with few conidial masses, setae absent in culture. Conidia 9.8–17.7 × 3.0–7.3 µm (mean = $15.1 \pm 2.0 \times 5.2 \pm 0.9$, n = 30), conidia smooth-walled, hyaline, and cylindrical with obtuse ends. Appressoria 7.3–12.2 × 4.9–9.3 µm (mean = $9.4 \pm 1.6 \times 6.2 \pm 1.2$, n = 20), formed from conidia, brown to dark brown, irregularly lobed in shape.

ADDITIONAL COLLECTIONS EXAMINED: CHINA. BEIJING, Shi Jingshan District, from leaves of *Populus nigra* var. *italica*, 25th October 2009, coll. C.M. Tian, HMBFU161, HMBFU141, HMBFU173 (BJFC, lyophilized PDA cultures).

PLATE 3

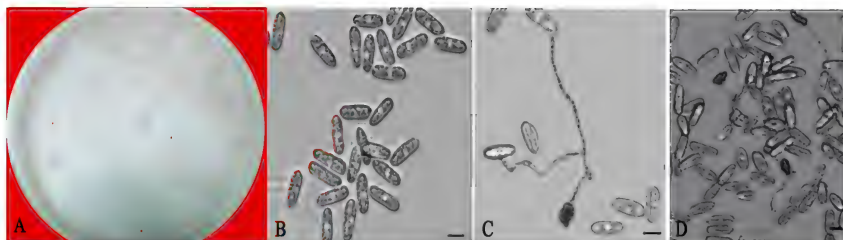


PLATE 3. *Colletotrichum populi* isolates (Group 2): cultural and morphological characters of holotype (isolate 1-9-1). A: Colony; B: Conidia; C & D: irregular appressoria. Bars = 10 μ m.

COMMENTS: *Colletotrichum populi* can be separated from the other species by both morphological and molecular characters (TABLE 5). The similar *C. asianum* grows more slowly and produces narrower conidia ($8.7\text{--}20.3 \times 3\text{--}4.7 \mu\text{m}$). The new species shares similar colony characteristics with *C. boninense*, *C. gloeosporioides*, and *C. kahawae*, all of which produce shorter and more rarely lobed appressoria. The genetically most closely related *C. fructicola* is distinguished by colonies that are grayish green in reverse with white edges. Colonies of *C. hymenocallidis*, which has similarly sized conidia and appressoria, appear denser in the central zone and produce fusiform conidia. *C. jasmine-sambac* differs by its colony with fimbriate margin.

Discussion

Both *Colletotrichum gloeosporioides* and *C. populi* have been found to cause anthracnose on poplars in Beijing region but can be distinguished by differing colony and appressoria morphologies as well as by molecular data derived from sequencing five genes. Appressorial size and shape have been used to distinguish some *Colletotrichum* species. For example, *C. gloeosporioides* was distinguished from *C. acutatum* by the shape of appressoria (Du et al. 2005). Crouch & Beirn (2009) pointed out that the size and shape of appressoria might be effective with the combination of host species during their work on anthracnose of cereals and grasses.

Although rDNA-ITS gene is not an ideal marker for intraspecific relationships, it is very useful in many cases for reconstruction of interspecific relationships (Cai et al. 2009). Other gene sequences have been examined in the phylogenetic analysis of *Colletotrichum*. GS and calmodulin (CAL) were found to be useful when studying anthracnose on coffee berries (Prihastuti et al. 2009). Compared with six gene regions, GPDH, CAL and ACT were useful for inferring the relationship of *C. gloeosporioides sensu lato* (Cai et al. 2009). Whilst there is no defined set of genes to be used for taxonomic studies of *Colletotrichum*, molecular data from a wider range of genes would give better insights into this group of fungi.

TABLE 5. *Colletotrichum populi* and closely related species.

SPECIES	COLONY	CONIDIA (µm)	APPRESSORIA (µm)	REFERENCE
<i>C. asianum</i>	Greenish white; in center greyish to dark green	Cyl. with obtuse ends, 8.7–20.3 × 3–4.7	Circular to sl. irregular, 3–5.1 × 2.1–3.3	Prihastuti et al. 2009
<i>C. boninense</i>	White aerial mycelium, reverse cream to orange	Cyl., 11.5–17 × 4–7	Irregularly shaped, 6–17 × 4–15	Jouji et al. 2003
<i>C. fructicola</i>	White; in age grey at the center	Cyl. with ends obtuse to sl. rounded, 8.5–16 × 3.5–5	Ovoid, occ. clavate, oft complex, 6–9 × 5.5–7	Prihastuti et al. 2009
<i>C. gloeosporioides</i>	Greyish white to dark grey, aerial mycelium	Cyl., base truncate, 12–17 × 3.5–6	Clavate, ovate, obovate, 6–20 × 4–12	Sutton 1992
<i>C. hymenocallidis</i>	White; later pale grey with circles	Fusiform, straight, ends obtuse 14–20 × 5–6.5	Ovate, occ. clavate, 7–11 × 5–7.5	Yang et al. 2009
<i>C. jasmine-sambac</i>	White aerial mycelium, with fimbriate margin	Cyl., straight with obtuse ends, hyaline, 13–15 × 3.5–4	Shape variable, ovoid, clavate, sl. irregular, brown	Wikee et al. 2010
<i>C. populi</i>	White to grey, aerial mycelium	Cyl., straight with obtuse ends, 9.8–22.0 × 2.9–7.3	Irregular, edge lobed, 7.3–12.2 × 4.9–9.3	This research
<i>C. kahawae</i>	Grey, later grey to dark, olive. grey	Straight, cyl., guttulate, apex obtuse, 7.5–17 × 3.5–5	Circular to sl. irregular, 4.5–10 × 7.5	Prihastuti et al. 2009

Acknowledgments

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***Sympodioplanus yunnanensis*, a new aquatic species
from submerged decaying leaves**GUANG-ZHU YANG, KAI-PING LU, YUE YANG, LI-BO MA,
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ABSTRACT — *Sympodioplanus yunnanensis* sp. nov. from submerged leaves collected from Gengma county, Lincang city, was found when researching aquatic hyphomycetes in Yunnan. The new species is well defined as a *Sympodioplanus* by its sympodial proliferation with multiple and crowded conidiogenous loci in the upper conidiophores and elongated conidia. It differs in conidial shape and septation from the type species, *S. capensis*.

KEY WORDS — anamorphic fungi, taxonomy

Introduction

Fungal diversity in southern China is high, and many anamorphic fungi collected in Yunnan Province have been published (Ma & Zhang 2007a,b,c, Shang & Zhang 2007, Wang & Zhang 2007, Ma et al. 2008, 2010, 2011, Zhang et al. 2009a,b,c, 2011a,b).

Sinclair et al. (1997) erected *Sympodioplanus* R.C. Sinclair & Boshoff for anamorphic fungi that exhibit sympodial proliferation with multiple and crowded conidiogenous loci in the upper part of the brown conidiophore, and elongated conidia with septa. Up to now, the monotypic genus comprised only *S. capensis* R.C. Sinclair & Boshoff isolated from dead decorticated wood. We add here a second species, *S. yunnanensis*, isolated from submerged decaying leaves.

Materials & methods

In 2010, a culture was isolated from leaves of a dicotyledonous plant submerged in a river in Yunnan Province, China. A 2 × 5 cm rotten leaf was spread on the surface of CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 mL distilled water) and incubated for ten days; single conidia were isolated using a sterilized

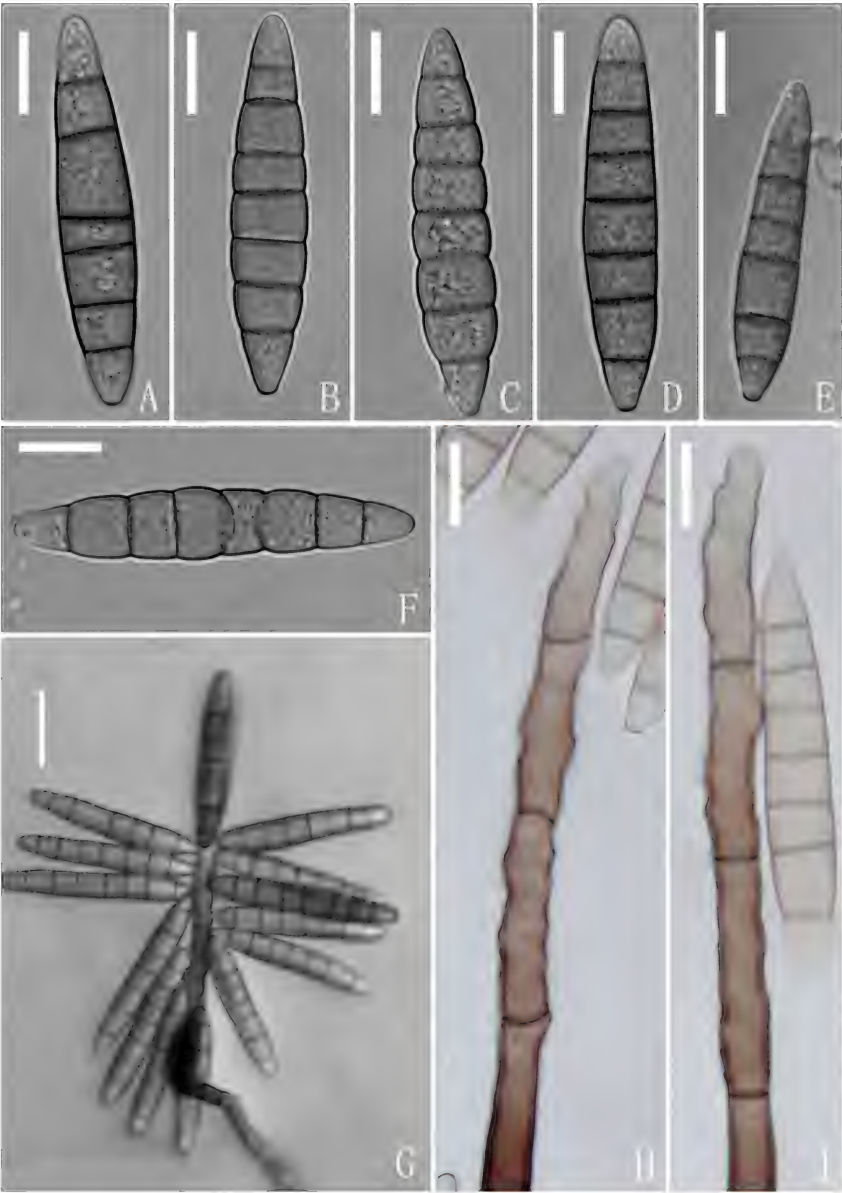


PLATE 1. *Sympodioplanus yunnanensis* (holotype: YMF1.03797). A–F. Conidia. G. Conidiophores bearing conidia. H–I. Conidiophores showing moderate protuberance in the apex region. Scale bars: A–F = 10 μ m, G = 25 μ m, H–I = 10 μ m.

toothpick viewed under a CX31 microscope and cultivated on CMA in Petri plates. Morphological observations were made from CMA after incubation at 25°C for one week; pure cultures and a permanent slide were deposited in the herbarium of Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF).

Taxonomy

Sympodioplanus yunnanensis G.Z. Yang & Z.F. Yu, sp. nov.

PLATE 1

MYCOBANK MB 563548

Differs from *Sympodioplanus capensis* by its larger conidia with more numerous septa.

TYPE: PR China, Yunnan Province, Lincang city, Meihua mountain, 23°38'N 99°22'E, elev. 1925 m, in a river on submerged leaves of an unidentified dicotyledonous plant, Dec 2010, G.Z. Yang (Holotype: YMF 1.03797; ex-type culture YMF 1.03797).

ETYMOLOGY: *yunnanensis* refers to the province in which the species was found.

Colonies effuse, brown, sparse, hairy, often inconspicuous, attaining 30 mm diam after 10 days on CMA at 25°C. Mycelium mostly immersed in the substrate, composed of branched, septate, hyaline to brown, smooth, 1.0–3.0 µm wide hyphae. Conidiophores macronematous, mononematous, solitary or in groups, erect, simple, straight, septate, brown, paler towards the apex, smooth, ≤ 70–205 µm long, 4.5–6.7 µm wide. Conidiogenous cells holoblastic, polyblastic, integrated and terminal, sympodial, thin-walled, bearing multiple, closely approximated protuberances at the upper cells, including the apex. Scars are flat in relation to the adjacent conidiogenous cell wall and not thickened, 0.5–1.0 µm wide. Conidia solitary, smooth, mostly spindle with truncate base, occasionally constricted at septa, at the apex mostly slightly attenuate, 38–56 × 6.0–10.8 µm, thin-walled, mostly 7-, sometimes 6-, 5-, or 4-septate.

COMMENTS: *Sympodioplanus yunnanensis* was easily determined as a *Sympodioplanus* by its similarity to the type species (*S. capensis*) in exhibiting sympodial proliferation with closely placed multiple conidiogenous loci in the upper conidiophore that are flat and equally thick as the conidiophore wall, forming solitary terminal and lateral conidia with cell walls similar to those of the upper conidiophore, and in producing elongated conidia with eusepta. However, the conidia of *S. capensis* are smaller (13–16(–19) × 2.5–4 µm) and have fewer septa (3(–5)-septate).

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***Phaeotrichoconis crotalariae*,
endophytic on *Vitis labrusca* in Brazil**

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ABSTRACT — *Phaeotrichoconis crotalariae* was isolated during studies with endophytic fungi on healthy leaves of *Vitis labrusca* in Pernambuco, Brazil. This is a new host for *P. crotalariae*, and the second report of this fungus from Brazil.

KEY WORDS — *Dematiaceae*, *Vitaceae*, taxonomy

Introduction

The genus *Phaeotrichoconis* was proposed by Subramanian (1956) after Salam & Rao (1954) reassessed *Trichoconis crotalariae* originally described on leaves of *Crotalaria verrucosa* L. The new genus was described as an anamorphic dematiaceous fungus with phragmosporic acrogenous brown elongated fusiform conidia with a long appendix.

Phaeotrichoconis crotalariae has a pantropical distribution in Africa, Asia, Australia, North America, and South America. It is reported to cause foliar flecks on *Acacia* spp. and has also been recorded on *Alternanthera*, *Chrysaliidocarpus*, *Cocos*, *Crotalaria*, *Cyperus*, *Elaeis*, *Marsilea*, *Oryza*, *Rotala*, and *Xanthium* (Chase 1982, Ellis 1971, Miller 1997, Mishra et al. 1972, Mohanan & Sharma 1988, Old et al. 1996, Ramakrishnan et al. 1972). In Brazil, it has been reported from Ceará State as a leaf endophyte of *Copernicia prunifera* (Freire & Bezerra 2001).

Phaeotrichoconis crotalariae was found in healthy leaves of *Vitis labrusca* in the Siriji Valley (municipalities of São Vicente Férrer and Macaparana), Pernambuco, Brazil. This paper presents a description of the fungus in culture.

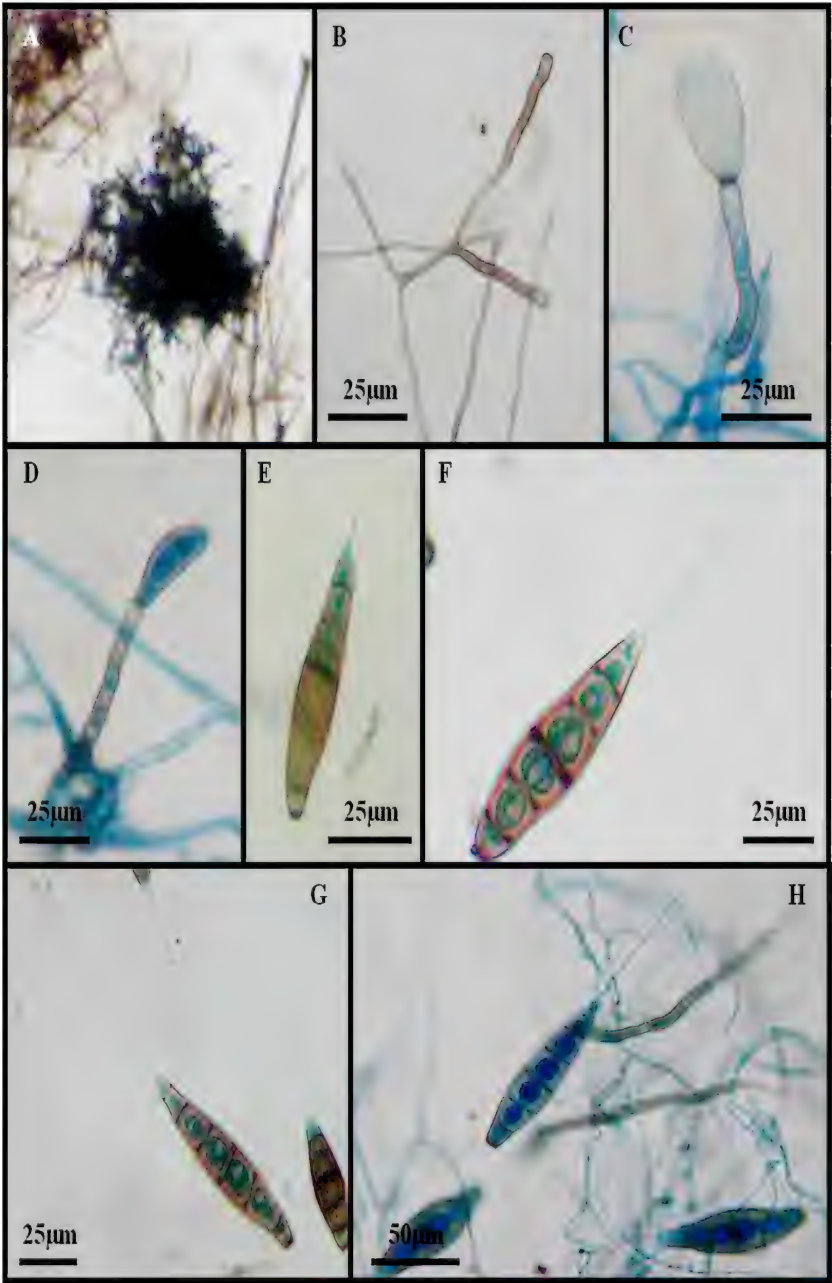


PLATE 1. *Phaeotrichoconis crotalariae*.
A. sclerotia; B. conidiophore; C–D. young conidia; E–H. mature conidia.

Materials & methods

During the February 2010 dry season, healthy mature leaves of *Vitis labrusca* were collected from forest areas of the municipalities of São Vicente Férrer and Macaparana, Pernambuco.

In the laboratory, each leaf was washed gently in running water with soap. Leaf discs were cut with a sterile metallic cork punch (6 mm diam.), decontaminated with 70% alcohol for 30 sec and hypochlorite sodium solution (NaOCl) at 2% for 2.5 min, and twice washed with sterilized distilled water in order to remove hypochlorite excess (Petrini 1996; modified technique). Six surface sterilized discs were transferred in triplicate to each Petri dish containing Potato Dextrose Agar (PDA) + chloramphenicol (50 mg.L⁻¹) to prevent bacterial growth. The plates were incubated at room temperature (28° ± 2°C) and observed daily during 15 days for colony development. For asepsis control, 50 µL of water used to remove hypochlorite was plated in PDA to confirm surface disinfection (Pereira et al. 1993). Species identification was based on macro- and micro-structural characteristics of the colony according to Subramanian (1956) and Ellis (1971). One isolated *P. crotalariae*, lyophilized and conserved in mineral oil, is maintained in the Culture Collection Depot at Universidade Federal de Pernambuco (URM).

Results

Five isolates of *P. crotalariae* were obtained from the dry season collection (February 2010).

Phaeotrichoconis crotalariae (M.A. Salam & P.N. Rao) Subram. Proc.

Indian Acad. Sci., B 44: 2 (1956)

PLATE 1

Colony on malt extract agar, incubated for eight days at room temperature (28 ± 2°C), showed well-developed, flocculate aerial mycelium, at first light orange, turning gray upon reaching maturity, reddish brown reverse. Conidiophores macronematous, mononematous, unbranched, straight or flexuous, brown, smooth, 19.2–141.6 × 3.6–4.8 µm. Polytretric conidiogenous cells, terminal, integrated, merged, sympodial, cylindrical, with dark scars. Conidia enteroblastic, acrogenous, solitary, dry, fusiform to obclavate, elongated, rostrate, usually with 5–6 transverse septa, thick walled, smooth, with a dark brown scar at the base, 45–79.2 × 12–17.5 µm. Appendix long, hyaline to pale brown, narrow (2.4 µm diam.), 35–220.8 µm long, 0–1 thin septum. Brown-dark sclerotia present.

SPECIMEN EXAMINED: BRAZIL. PERNAMBUCO: SÃO VICENTE FÉRRER, in healthy mature leaves of *Vitis labrusca* L. cv. Isabel, 11 Feb 2010, Lima (URM 6360 [culture conserved in mineral oil and lyophilized]).

NOTES: This is the second record of *Phaeotrichoconis crotalariae* from Brazil, and the first time it has been described on artificial culture medium. The fungus has not previously been recorded from *Vitis*.

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**Studies on *Wrightoporia* from China 1.
A new species from Hunan Province, South China**

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ABSTRACT — *Wrightoporia nigrolimitata* sp. nov. is described from Hunan Province, southern China. It is characterized by an annual growth habit, small pileate basidiocarps, a brown to black line between the tubes and context, a monomitic hyphal system with clamp connections, nondextrinoid hyphae, and finely asperulate amyloid basidiospores. A key to accepted Chinese *Wrightoporia* species is supplied.

KEY WORDS — *Bondarzewiaceae*, polypore, taxonomy, wood-inhabiting fungi

Introduction

Pouzar (1966) established *Wrightoporia* Pouzar for *W. lenta* (Overh. & J. Lowe) Pouzar, the type species. This genus is characterized by resupinate to pileate basidiocarps, annual to perennial growth habit, monomitic to trimitic hyphal system, and amyloid asperulate basidiospores (Pouzar 1966, Ryvarden 1982). Of the 39 species described in or transferred to the genus worldwide (Buchanan & Ryvarden 2000; David & Rajchenberg 1987; Hattori 2003, 2008; Johansen & Ryvarden 1979; Lindblad & Ryvarden 1999; Loguercio-Leite et al. 1998; Núñez & Ryvarden 2001; Rajchenberg & David 1990; Ryvarden 1975, 1987, 2000), 14 species have been found in China (Cui & Dai 2006; Dai 2012; Dai & Cui 2006; Dai et al. 2011).

Many new polypores have been recently described due to research on the diversity of wood-inhabiting fungi in southern China (Cui & Dai 2008, 2011; Cui et al. 2009, 2010, 2011abc; Dai et al. 2010, 2011; Du & Cui 2009; Jia & Cui 2011; Li & Cui 2010). During our study of wood-rotting fungi from Mangshan Forest Park, Hunan Province, we found one *Wrightoporia* species with a monomitic hyphal structure that cannot be identified to any known species. We describe it as a new species in this paper and provide an identification key to the 15 *Wrightoporia* species recorded in China.

Materials & methods

Sections were studied microscopically according to Dai (2010) at magnifications $\leq 1000\times$ using a Nikon Eclipse E 80i microscope with phase contrast illumination. Drawings were made with the aid of a drawing tube. To present spore size variation, the 5% of measurements excluded from each end of the range are given in parentheses. Basidiospore spine lengths are not included in the measurements. Abbreviations include IKI = Melzer's reagent, IKI- = negative in Melzer's reagent, KOH = 5% potassium hydroxide, CB = Cotton Blue, CB- = acyanophilous, L = mean spore length (arithmetic average of all spores), W = mean spore width (arithmetic average of all spores), Q = variation in the L/W ratios between the specimens studied, and n = number of spores measured from given number of specimens. Special colour terms follow Petersen (1996). The studied specimens were deposited in herbaria as cited below.

Taxonomy

Wrightoporia nigrolimitata Jia J. Chen, sp. nov.

FIG. 1

MYCOBANK MB 564036

Differs from other *Wrightoporia* species by its small pileate basidiocarps, presence of a black layer between tubes and context, a monomitic hyphal system, and generative hyphae that are nondextrinoid and dissolve in KOH.

TYPE — China. Hunan Province, Yizhang County, Mangshan Forest Park, on angiosperm stump, 25.VI.2007, Li 1697 (holotype, BJFC; isotype, IFP).

ETYMOLOGY — *nigrolimitata* (Lat.): referring to the black line separating the tubes and context.

FRUITBODY — Basidiocarps annual, pileate, hard corky upon drying, without odor or taste; pileus semi-circular to irregularly formed, projecting up to 1 cm long, 2 cm wide, 4 mm thick at base. Pileal surface straw-yellow to honey-yellow at base, buff-yellow towards the margin, azonate, slightly sulcate or not; margin obtuse. Pore surface cream-buff to pinkish buff when dry; pores round to angular, 4–7 per mm; dissepiments thin to slightly thick, entire. Context buff to cinnamon-buff, hard corky, up to 1 mm thick. Tubes concolourous with pore surface, fibrous-tough, up to 3 mm long; a brown to black line present between tubes and context.

HYPHAL STRUCTURE — Hyphal system monomitic; generative hyphae bearing clamp connections, IKI- or weakly dextrinoid in the black layer, CB-, hyphae in trama and context dissolved in KOH; hyphae in the black layer become reddish to dark brown in KOH.

CONTEXT — Generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, regularly arranged, 1.5–8 μm in diam.

TUBES — Generative hyphae hyaline, thin- to slightly thick-walled, frequently branched, subparallel to parallel along the tubes, 2–7 μm in diam; cystidia absent, fusoid cystidioles present, hyaline, thin-walled, 13–19 $\times$ 3.5–5 μm ; basidia clavate, bearing four sterigmata and a basal clamp connection,

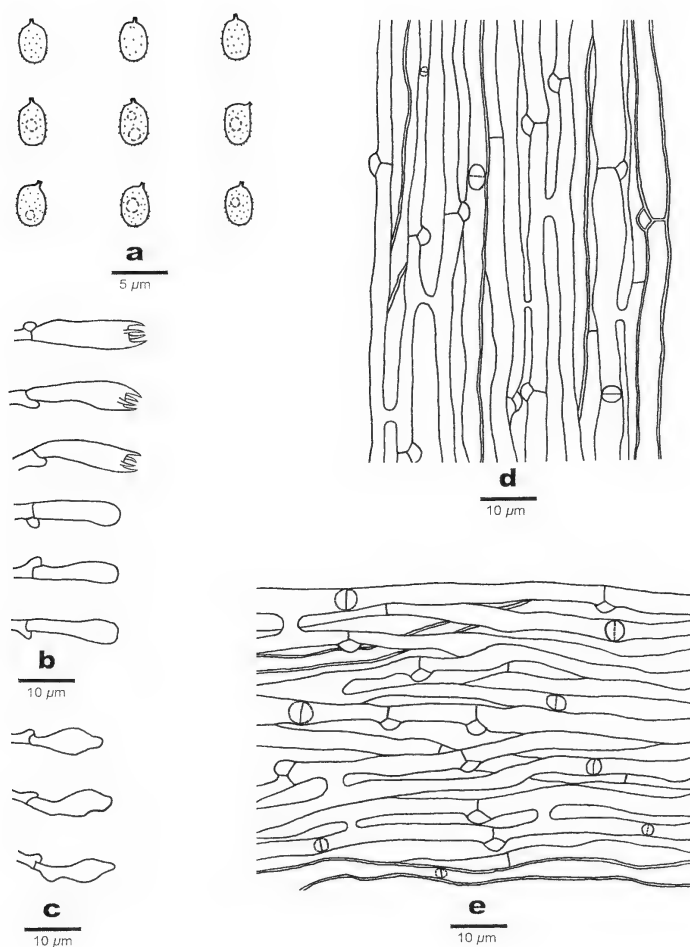


FIG. 1. Microscopic structure of *Wrightoporia nigrolimitata* (drawn from the holotype).

a: Basidiospores. b: Basidia and basidioles. c: Cystidioles.

d: Hyphae from trama. e: Hyphae from context.

13–25 × 4–6 μm; basidioles in shape similar to basidia, but slightly smaller.

SPORES — Basidiospores ellipsoid, hyaline, thin-walled, mostly bearing one or two small guttules, finely asperulate, **amyloid**, CB–, 3–4 × 2–2.7(–2.9) μm, L = 3.43 μm, W = 2.42 μm, Q = 1.42 (n = 30/1).

REMARKS — Although most *Wrightoporia* species possess a dimitic hyphal system, only three others have a monomitic hyphal system: *W. flava* (Ryvarden)

A. David & Rajchenb., *W. novae-zelandiae* Rajchenb. & A. David, and *W. porilacerata* Log.-Leite et al. (David & Rajchenberg 1987, Loguercio-Leite et al. 1998, Rajchenberg & David 1990, Ryvarden 1975).

Wrightoporia porilacerata also possesses pileate basidiocarps and similar sized ($3-3.5 \times 2-2.5 \mu\text{m}$) basidiospores but is distinguished from *W. nigrolimitata* by distinctly larger basidiocarps ($\leq 13.5 \times 8.7 \times 2.6 \text{ cm}$), larger pores (1–3 per mm), no black line between tubes and context, and interwoven tramal hyphae (Loguercio-Leite et al. 1998).

Like *W. nigrolimitata*, *W. flava* has pileate basidiocarps, nondextrinoid generative hyphae, and thin-walled asperulate amyloid basidiospores. However, *W. flava* is distinguished by possessing gloeoplerous hyphae, interwoven tramal hyphae, and lack of a black layer (David & Rajchenberg 1987, Ryvarden 1975).

Except for a monomitic hyphal system, *W. novae-zelandiae* is unique in *Wrightoporia* by combining a gossypine brittle resupinate basidiocarp with gloeoplerous hyphae (Rajchenberg & David 1990).

OTHER SPECIMENS EXAMINED — *Wrightoporia flava*. GABON. OGOOUÉ-IVINDO PROVINCE, Makokou, Ipassa, XII.1974, LY 1733 (LY). TANZANIA. TANGA PROVINCE, Usambara Mts. Amani, Tanga distr., 18.II.1973, Ryvarden 10594 (O). MALAWI. SOUTHERN PROVINCE, Makwawa, 16.XII.1981, B.M. 342 (O).

— *W. novae-zelandiae*. NEW ZEALAND. AUCKLAND, Waitakere Ranges, Kitekite Track, on *Leptospermum* sp., 19.IV.1989, P.K. Buchanan 89/114 (paratype, PDD 55206).

— *W. porilacerata*. BRAZIL. PARANA STATE, Parangua, Guaraguacu, fazenda Sambaqui, on decayed dicotyledon trunk, 22.VI.1993, A.A.R. De Meijer 2805 (O).

Key to species of *Wrightoporia* in China

- 1. Hyphal system monomitic *W. nigrolimitata*
- 1. Hyphal system dimitic or trimitic 2
- 2. Generative hyphae without clamp connections 3
- 2. Generative hyphae with clamp connections 5
- 3. Basidiocarps pileate; basidiospores $>7 \mu\text{m}$ long . . . *W. radicata* G.Y. Zheng & Z.S. Bi
- 3. Basidiocarps resupinate to effused-reflexed; basidiospores $<7 \mu\text{m}$ long 4
- 4. Basidiocarps without rhizomorphs *W. casuarinicola* Y.C. Dai & B.K. Cui
- 4. Basidiocarps with rhizomorphs *W. rubella* Y.C. Dai
- 5. Basidiospores $>5.3 \mu\text{m}$ long *W. lenta*
- 5. Basidiospores $<5.3 \mu\text{m}$ long 6
- 6. Skeletal hyphae indextrinoid 7
- 6. Skeletal hyphae dextrinoid 8
- 7. Cystidia and gloeocystidia absent *W. aurantipora* T. Hatt.
- 7. Cystidia and gloeocystidia present *W. austrosinensis* Y.C. Dai
- 8. Gloeoplerous hyphae present 9
- 8. Gloeoplerous hyphae absent 10

9. Basidiocarps membranous; tramal skeletal hyphae 1–2 µm in diam *W. avellanea* (Bres.) Pouzar
9. Basidiocarps tough; tramal skeletal hyphae 2–2.5 µm in diam .. *W. borealis* Y.C. Dai
10. Basidiospores >3.2 µm wide *W. unguliformis* Y.C. Dai & B.K. Cui
10. Basidiospores <3.2 µm wide 11
11. Skeletal hyphae not encrusted 12
11. Skeletal hyphae encrusted 13
12. Basidiocarps annual; tramal skeletal hyphae 2–4 µm in diam *W. africana* I. Johans. & Ryvarden
12. Basidiocarps perennial; tramal skeletal hyphae 4–8 µm in diam *W. tropicalis* (Cooke) Ryvarden
13. Skeletal hyphae in the trama inflated, up to 6–9 µm . *W. luteola* B.K Cui & Y.C. Dai
13. Skeletal hyphae in the trama not inflated, up to 5 µm 14
14. Generative hyphae with both clamp connections and simple septa; cystidioles absent *W. gillesii* A. David & Rajchenb.
14. Generative hyphae only with clamp connections; fusoid cystidioles present *W. japonica* Núñez & Ryvarden

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A new species of *Bipolaris* from IranABDOLLAH AHMADPOUR^{1*}, ZEINAB HEIDARIAN¹, MARYAM DONYADOOST-CHELAN¹, MOHAMMAD JAVAN-NIKKHAH¹ & TAKAO TSUKIBOSHI²

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ABSTRACT— *Bipolaris salkadehensis* sp. nov., isolated from the leaves of *Sparganium erectum* (Thyphaceae) and *Cladium mariscus* (Cyperaceae) collected in Iran, is described and illustrated. The new species is characterized by straight to slightly curved subcylindrical to fusoid conidia with end cells demarcated by thick dark septa and with monopolar and bipolar germination. Morphological data and ITS+5.8SrDNA sequence analyses support *B. salkadehensis* as a distinct species of *Bipolaris*.

KEY WORDS — morphology, taxonomy, fungus, *Cochliobolus*, phylogeny

Introduction

Bipolaris species are known to infect many kinds of grasses causing spots or flecks on leaves or stems and sometimes cause diseases on herbaceous plants such as *Cactaceae* and *Musaceae* (Ellis 1971, Sivanesan 1987). Since Sivanesan (1987) monographed the graminicolous species of the genus, several new *Bipolaris* species have been described (Chiang et al. 1989, Peng & Lu 1989, Sisterna 1989, Alcorn 1990, Sivanesan 1992, Alcorn 1996, Chen et al. 2000, Zhu et al. 2000, Deng & Zhang 2002, Jiang & Zhang 2008, Zhang & Li 2009). Recent molecular phylogenetic analyses have shown that *Bipolaris* and *Curvularia* are closely related to each other and comprise a different clade from *Exserohilum* (Berbee et al. 1999, Olivier et al. 2000). The rDNA-ITS sequences of (particularly the internally transcribed spacers ITS1 and ITS2) are useful tools for resolving taxonomic relationships within *Bipolaris*.

In this study, we describe a new species of *Bipolaris*, which we compare both morphologically and phylogenetically with other species.

Materials & methods

Collection and isolation of the fungus

Plant material was obtained from the Salkadeh village, Khoys city, West Azerbaijan province, in northwestern Iran in the summer of 2010. Leaf samples of *Sparganium erectum* L. (*Thyphaceae*) and *Cladium mariscus* (L.) Pohl (*Cyperaceae*) with brown oval to elliptical lesions with approximately 2–3 mm in length were collected and stored dry in a refrigerator at 5°C until use. A single spore produced on the surface-sterilized leaf under NUV light on 12 h diurnal cycle at room temperatures $23 \pm 2^\circ\text{C}$ (Sivanesan 1987) was transferred to tap water agar with autoclaved wheat straw (TWA+wheat straw) plates. Five isolates were obtained: Bi-1–3 from *S. erectum*, and Bi-4–5 from *C. mariscus*. For morphological inspection each isolate was transferred to Petri dishes containing PDA using a thin glass needle and incubated at $23 \pm 2^\circ\text{C}$ under darkness; species descriptions are based on 10–14 day old cultures. Measurement and microphotographs were taken from slide mounts in lactophenol and lactophenol cotton blue using an Olympus light microscope (model BH2). The fungal isolates were compared with the descriptions of Ellis (1971, 1976), Sivanesan (1987) and reported species after 1987. Dried culture vouchers have been deposited in the herbarium in the Department of Plant Protection, Faculty of Agricultural Sciences & Engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran (TUPP).

Molecular phylogenetic analyses

Whole genomic DNA was extracted from the mycelium of each isolate grown on V8 juice agar by homogenization in a standard sodium dodecyl sulfate (SDS) detergent lysis buffer followed by a phenol : chloroform extraction and precipitation in ethanol with sodium acetate (Sambrook et al. 1989). The ITS1+5.8S+ITS2 rDNA region was amplified using the polymerase chain reaction (PCR) conditions and the ITS1 and ITS4 primer pair (White et al. 1990). Purified PCR products were sequenced by ABI PRISM 3100 automated sequencers (Applied Biosystems, Foster City, CA, USA). For phylogenetic comparison, the GenBank sequences of 26 *Bipolaris* species, 5 *Curvularia* species, 2 *Exserohilum* species, 2 *Drechslera* species, and *Alternaria alternata* (Fr.) Keissl. (as outgroup) were included (Berbee et al. 1999). The DNA sequences were aligned using Clustal X version 1.8 (Thompson et al. 1997). Further visual alignments were done in Sequence Alignment (Se-Al) Editor version 2.0 (Rambaut 2000). The data were analysed phylogenetically using distance methods. The distance matrix was calculated using Kimura's two parameter method (Kimura 1980) and analyzed with the neighbor-joining (NJ) method (Saitou & Nei 1987) using the program PAUP* 4.0 beta 10 (Swofford 2002). Bootstrap values were generated with 1000 replicate heuristic searches to estimate support for clade stability of the consensus tree using the same program. Sequences have been deposited in the DNA Data Bank of Japan (DDBJ).

Results

New species

Bipolaris salkadehensis Ahmadpour & Heidarian, sp. nov.

FIG. 1

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Differs from *Bipolaris cynodontis* in conidia with both monopolar and bipolar germination and conidial end cells that are demarcated by thick dark septa.

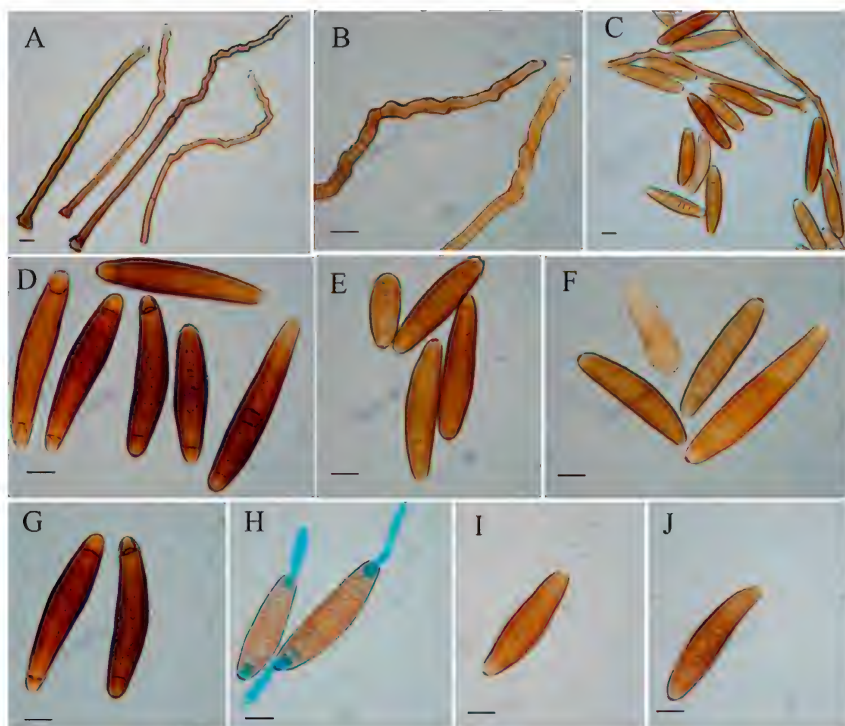


FIG. 1. *Bipolaris salkadehensis*. A, B. Conidiophores. C–G. Conidia. H–J. Germinating conidia. Scale bars = 10 µm.

TYPE: Iran. West Azerbaijan, Khoy City, Salkadeh village, on infected leaves of *Sparganium erectum*, 20 Sep 2010, coll. A. Ahmadi-pour Bi-1 (Holotype, TUPP1366 [dried culture]; DDBJ sequence, AB675490).

ETYMOLOGY: referring to the type locality.

Colonies in 'TWA+wheat straw' agar brown, velvety, floccose. Hyphae brown, septate, branched, smooth, 5–7 µm wide. Stromata not formed. Conidiophores single or fasciculate in small groups, simple, pale brown to brown, cicatrized with scars often inflated and smooth, multiseptate, 225–590 µm long, swollen to 7.5–15 µm diam at base, then narrowing to 5–8.5 µm diam (middle) and 5–7.5 µm diam (apex). Conidia pale brown to dark brown, subcylindrical to fusoid, occasionally obclavate to clavate, straight to slightly curved, smooth, end cells rounded, very pale and demarcated by thick, dark septa, (4–)6–8 (–10) distoseptate, (32–)53–80(–93) × 12–15 µm, hilum ≤ 2 µm diam, sometimes slightly protruding. Germination of conidia is monopolar and bipolar. The primary septum in developing conidia is submedian, the second delimiting the basal cell, and the third distal.

Cultural characteristics: Colony velvety, floccose, olivaceous-brown on PDA.

ADDITIONAL MATERIAL EXAMINED: IRAN. WEST AZERBAIJAN, Khoy City, Salkadeh village, on infected leaves of *Cladium mariscus*, 20 Sep 2010, coll. A. Ahmadpour Bi-4 (TUPP1362; DDBJ sequence, AB675491) and Bi-5, on infected leaves of *Sparganium erectum*, Bi-2–3.

COMMENTS: Its conidial morphology clearly establishes our specimen as belonging to *Bipolaris*. It is morphologically similar to *B. cynodontis*, *B. setariae* (Sivanesan 1987), *B. sesuvii* (Zhang & Li 2009), and *B. fusca* (Jiang & Zhang 2008). *Bipolaris cynodontis* is distinguished from the new species by its smaller conidia, absence of thick dark septa demarcating the end cells demarcated by thick dark septa, and with only bipolar germination (Sivanesan 1987). The conidia of *B. setariae* are somewhat similar — fusoid to navicular, pale to mid golden brown, 5–10 distoseptate, (45–)50–70(–100) × 10–15 µm (Sivanesan 1987) — but with end cells demarcated by unthickened hyaline septa and paler than those of *B. salkadehensis*. The conidia of *B. sesuvii* are olivaceous-brown to brown, basal cell concolorous or slightly pale, subcylindrical to broadly fusoid and rounded at ends, straight, smooth, 5–9 distoseptate, 52–77 × 13–16 µm (Zhang & Li 2009) and are narrower and have end cells demarcated by unthickened hyaline septa. The conidia of *B. fusca*, which are shorter and wider than those of *B. salkadehensis*, are straight or occasionally slightly curved, smooth, cylindrical or ellipsoidal, (3–)5–7(–11) distoseptate, 31–67 × 11–20 µm, yellowish brown to dark brown, with end cells often very pale and demarcated by thick dark septa (Jiang & Zhang 2008).

Phylogenetic analysis & discussion

PCR products of ITS+5.8S rDNA sequences were 506bp for isolate Bi-1 and 505bp for isolates Bi-2–5. Since the Bi-2–5 sequences matched completely, the Bi-1 sequence was registered as DDBJ AB675490 and the Bi-4 as DDBJ AB675491. According to the neighbor-joining tree derived from ITS+5.8S rDNA sequences made in this study, the *B. salkadehensis* isolates formed a monophyletic group with a 95% bootstrap value close to *B. cynodontis* (AF163093) (FIG. 2). However, the 98.2% (497/506 bp) sequence similarity between *B. salkadehensis* and *B. cynodontis* indicates that those two species clearly differ. Moreover, despite similar conidial morphologies, *B. salkadehensis* formed a different group from *B. setariae* and *B. sesuvii* (FIG. 2).

Berbee et al. (1999) used the ITS+5.8S rDNA sequences and a portion of the glyceraldehyde-3-phosphate dehydrogenase sequences to evaluate *Cochliobolus* (anamorphs *Bipolaris*) and proposed that *Bipolaris* be divided further, separating species with large, canoe-shaped, gently curving conidia (*Cochliobolus* Group 1) from those with short, either straight or curved conidia lacking a gentle curve along the whole spore length (*Cochliobolus* Group 2), which intermix

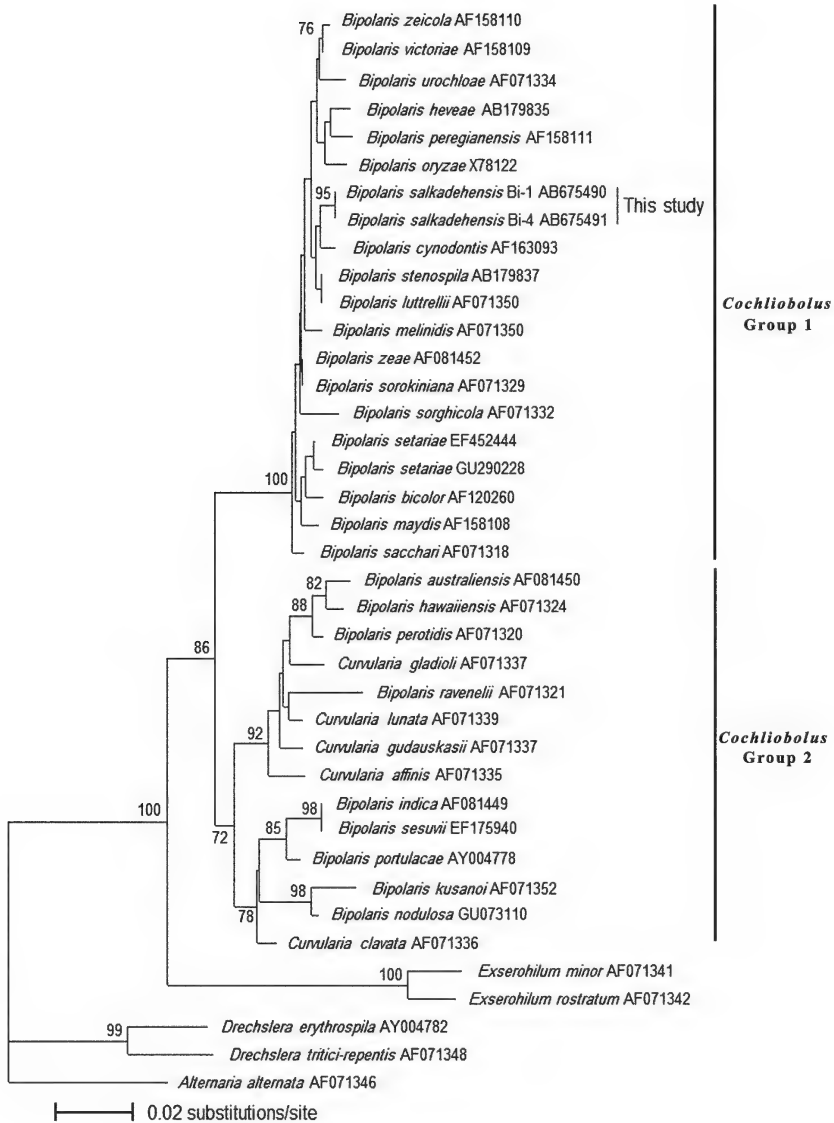


FIG. 2. A neighbor-joining tree inferred from the ITS+5.8S rDNA sequences from 35 taxa. Bootstrap values for 1000 replicates are shown on the branches, and rDNA-ITS DDBJ accession numbers stand after the species names. The branch length is proportional to the number of base changes as indicated by the scale bar. *Alternaria alternata* (AF071346) is an outgroup.

with *Curvularia* species. All *B. salkadehensis* isolates used in this study clustered in the *Cochliobolus* Group 1 subclade, which contains *Bipolaris* species large, canoe-shaped, gently curving conidia. Although the conidial morphology of *B. salkadehensis* is similar to *B. cynodontis*, *B. setariae* (*Cochliobolus* Group 1), and *B. sesuvii* (*Cochliobolus* Group 2), the phylogenetic analysis did not show a close relationship. The combined molecular-morphological analysis supports *B. salkadehensis* as an unreported new species.

Although the teleomorph was not observed in cultures or on the natural host, the *B. salkadehensis* teleomorph relationships can be compared by using differences or similarity in conserved DNA sequences (e.g. ITS, GPD, BRN1 and mating type genes) with other known *Bipolaris* species to provide a more reliable classification system at the generic and species levels (Berbee et al. 1999, Shimizu et al. 1998, Turgeon 1998). The MAT phylogenetic trees, along with the ITS and GPD trees, can be used as a database from which to look back into evolutionary history in order to understand how different reproductive lifestyles arose (Turgeon 1998).

Both morphological and phylogenetic evidence supports *B. salkadehensis* as a previously unreported species separate from other known *Bipolaris*. This is the first report of *Bipolaris* on the hosts *S. erectum* (*Thyphaceae*) and *C. mariscus* (*Cyperaceae*).

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Notes on *Xylophallus xylogenus* (Phallaceae, Agaricomycetes) based on Brazilian specimens

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ABSTRACT — From morphological analysis of *Phallus pygmaeus* specimens collected in Brazil and reexamination of the holotype, we conclude that this species is a synonym of the previously described *Xylophallus xylogenus* and agree with the recently proposed synonymy. We describe new specimens of *X. xylogenus* from Northeastern Brazil and provide color photos and a key for the species of the small xylophilous members of *Phallus*, including *Xylophallus*.

KEY WORDS — gasteromycetes, herbarium revision, *Mutinus*, stinkhorns, tropical fungi

Introduction

Phallus pygmaeus was described from the tropical rainforest in Northeastern Brazil, growing on decaying wood (Baseia et al. 2003). The species is characterized by small basidiomata (≤ 15 mm high), a smooth receptacle with minute perforate apex, and lignicolous habitat. The remarkably small size of this phalloid is not exclusive to *P. pygmaeus*, as other small species are known from the tropics, e.g., *Phallus tenuis* (E. Fisch.) Kuntze, *P. minusculus* Kreisel & Calonge, and *P. drewesii* Desjardin & B.A. Perry (Calonge & Kreisel 2002, Calonge 2005, Desjardin & Perry 2009).

Xylophallus xylogenus is a taxon with a problematic generic position. Originally described as *Phallus xylogenus* from Cayenne (French Guiana), its author (Montagne 1855) placed the species in sect. *Mutinus*, while some years later Schechtendal (1861) classified it in *Phallus* sect. *Xylophallus* Schltld. Fischer (1898-99), who first proposed the combination *Mutinus xylogenus*, later erected the genus *Xylophallus* (Schltld.) E. Fisch. for the species (Fischer 1933a). Sáenz et al. (1972) also contributed to the morphology and ontogeny of the species based on collections from Costa Rica. Since its original description, *X. xylogenus* has been ambiguously illustrated by different authors (Fig. 1).

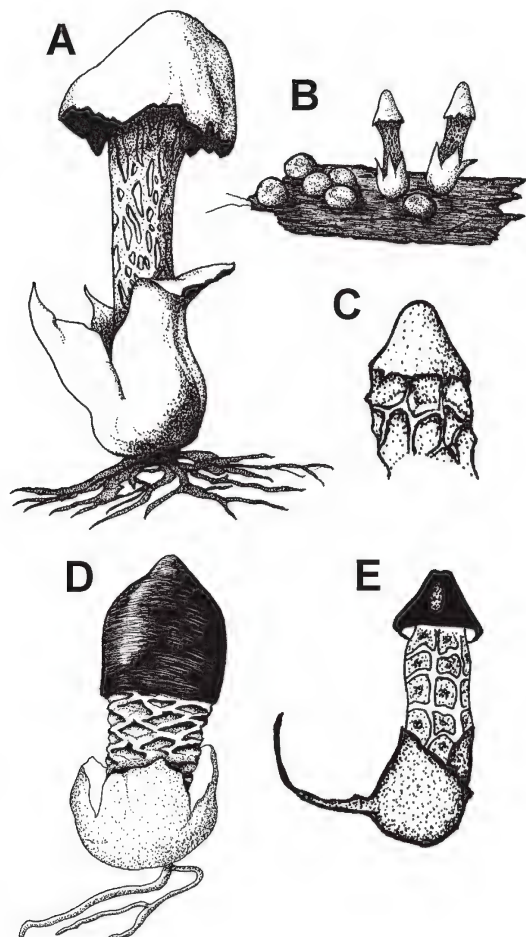


FIG. 1. *Xylophallus xylogenus*, redrawn from original publications:
A–B. Montagne (1855), as *Phallus xylogenus*. C. Fischer (1933).
D. Sáenz et al. (1972). E. Baseia et al. (2003), as *Phallus pygmaeus*.

Cheyne (2010), who recently described and provided colour photos of *X. xylogenus* specimens from the type locality, proposed synonymizing *P. pygmaeus* with *X. xylogenus*.

During field expeditions in the State of Pernambuco, Northeastern Brazil, specimens initially determined as *P. pygmaeus* were collected in abundance

from rotten logs. Our analysis of this material and reexamination of the *P. pygmaeus* holotype leads us to confirm Cheype's (2010) synonymy.

Materials & methods

Field expeditions were carried out in 2008 and 2009 in two reserves in the State of Pernambuco, Northeast Region of Brazil: Reserva Privada do Patrimônio Natural (RPPN) Carnijó (Moreno; 8°10'00"S 35°05'15"W) and Parque Dois Irmãos (Recife; 8°07'30"S 34°52'30"W). Both reserves, at altitudes at 20–180 m, are relict fragments of the Brazilian Atlantic rainforest. The sampled basidiomata were transported to the laboratory in plastic boxes (Lodge et al. 2004). Both fresh and dried basidiomata were examined.

Macroscopic measurements and colors are based on fresh material. Colors are coded according to Kornerup & Wanscher (1978). Observations of microscopic characters were made under a light microscope on glass slides mounts (in 5% KOH) prepared from dried specimens. Voucher specimens are deposited in URM (Thiers 2011).

Taxonomy

Xylophallus xylogenus (Mont.) E. Fisch., Nat. Pflanzenfam., 2 Aufl., 7a: 96. 1933.

Figs 1–3

= *Phallus (Mutinus) xylogenus* Mont., Ann. Sci. Nat., Bot., Sér. 4, 3: 137. 1855.

= *Mutinus xylogenus* (Mont.) E. Fisch., Nat. Pflanzenfam. 1(1**): 290. 1899.

= *Phallus pygmaeus* Baseia, Mycotaxon 85: 78. 2003.

BASIDIOMATA gregarious; when immature globose, subglobose to ovoid, sometimes tapering towards the base, glabrous, smooth or groovy, 2.5–3.5 mm high × 2.5–4 mm broad, yellowish brown (5D5, 5E5) at the apex, becoming lighter near to the base; at maturity, 6–14 mm high, including the volva. **VOLVA** yellowish brown, with irregular dehiscence, basal hyphal strand simple or multiple, whitish, penetrating the woody substrate or forming a network above it. **PSEUDOSTIPE** cylindrical, hollow inside, 5–7 mm high × 1.5–2.5 mm broad, translucent white, indusium absent; externally ornamented by a reticulated structure composed of rectangular alveoles with prominent edges. **RECEPTACLE** conic-campanulate, surface smooth, adnate to the pseudostipe, with round or umbilicate apex, non or minutely perforate at maturity, 2.5–4 mm high × 2.5–4 mm broad, concolor with pseudostipe; receptacle margin smooth to denticulate. **GLEBA** gelatinous, grayish green (1D5) to olive (1F7), very foetid.

BASIDIOSPORES ellipsoid, smooth, thin-walled, hyaline or with greenish tints, 3–4 × 1.5 µm. **VOLVA** composed of isodiametric to irregularly rectangular pseudoparenchymatous hyphae, hyaline, yellowish to brownish, slightly thick-walled, 7–15 × 6–13 µm. **PSEUDOSTIPE** AND **RECEPTACLE** composed by isodiametric pseudoparenchymatous hyphae, hyaline, thin-walled or slightly thick-walled, 12–45 × 12–32 µm.

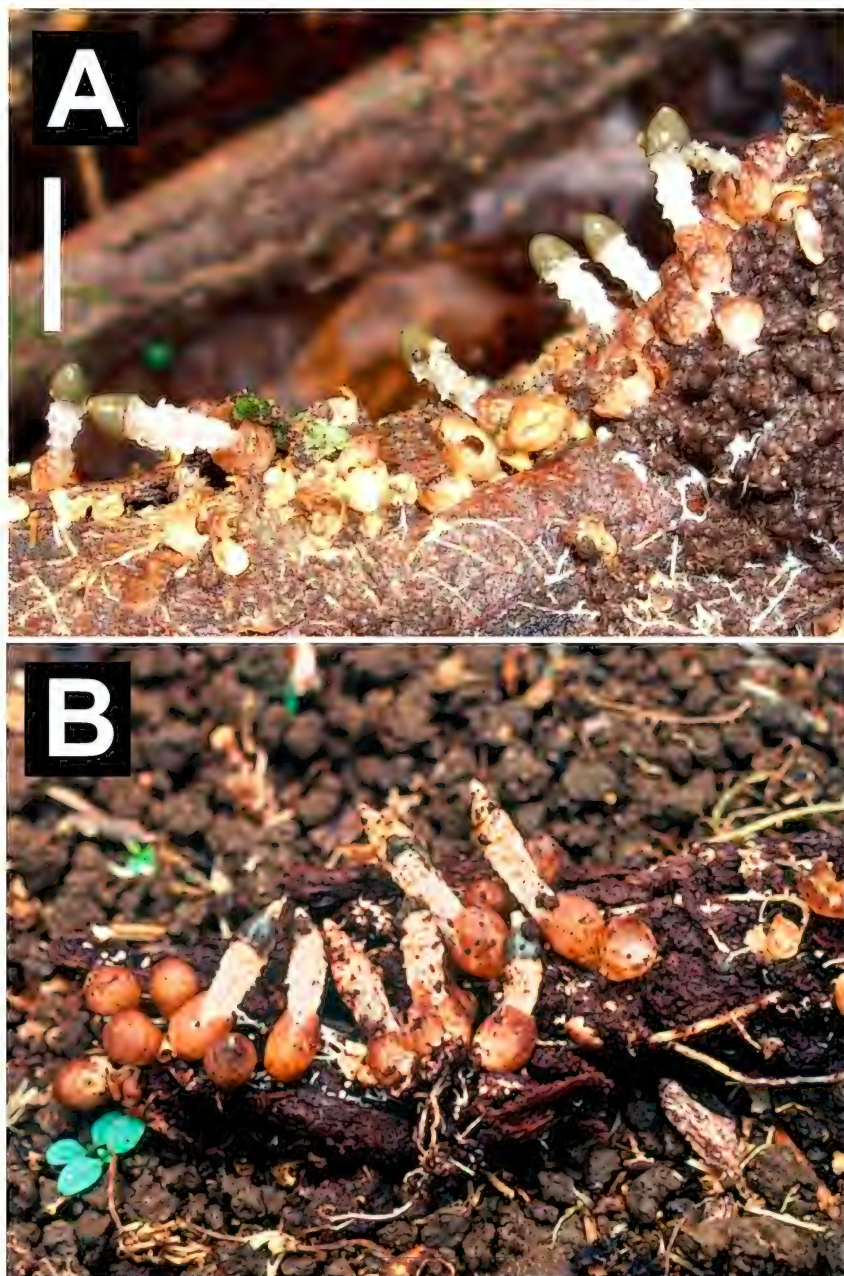


FIG. 2. *Xylophallus xylogenus* in situ.

A. Brazilian basidiomata (scale = 12 mm). B. Costa Rican basidiomata (photo by Clark L. Ovrebo).

ECOLOGY & DISTRIBUTION: on rotten wood in French Guiana (Montagne 1855, Cheype 2010), French Antilles (Guadeloupe; Cheype 2010), Suriname (Fischer 1933b), Costa Rica (Sáenz et al. 1972, Sáenz & Nassar 1982, Calonge et al. 2005), Brazil (as *P. pygmaeus*; Baseia et al. 2003, Baseia et al. 2006, Leite et al. 2007), Peru and Ecuador (Gómez & Gazis 2006).

SPECIMENS EXAMINED: **BRAZIL. PERNAMBUCO,** GURJAÚ, 28.VI.2002, leg. Baseia & Gibertoni (URM 77078, holotype of *Phallus pygmaeus*); **MORENO,** RPPN Carnijó, 17.VI.2008, leg. Trierweiler-Pereira 10, 11 (URM 80261, 80262); 08.VII.2008, Trierweiler-Pereira 83, 84 (URM 80264, 80265); 14.VIII.2008, Trierweiler-Pereira 186, 187 (URM 80269, 80270); 23.IX.2008, Trierweiler-Pereira 148, 149 (URM 80267, 80268); 16.X.2008, Trierweiler-Pereira (URM 80271); 23.I.2009, Baltazar & Coimbra 206 (URM 80272); 12.III.2009, Trierweiler-Pereira 207, 208 (URM 80273, 80274); 21.V.2009, Trierweiler-Pereira 226, 227 (URM 80275, 80276); **RECIFE,** Parque Dois Irmãos, 07.VII.2008, Trierweiler-Pereira 67 (URM 80263); 12.VIII.2008, Trierweiler-Pereira 142 (URM 80266).

REMARKS: Desjardin & Perry (2009) noted that *P. xylogenus* was a long forgotten name. Various authors have illustrated it differently (FIG. 1). Montagne's (1855) illustration (FIG. 1B), which is representative, depicts its xylophilous gregarious habit, while Montagne's detailed figure of a single basidiome (FIG. 1A) is quite imaginary, showing the receptacle margin distant from the pseudostipe and the pseudostipe with a shallow reticulum. The illustration in Fischer (1933a), based on original material, is more realistic and the pseudostipe with deep alveoles is well represented (FIG. 1C).

Lloyd's (1907) photo of the original material shows that the upper basidiome of the type was not well preserved, leading him to describe the species as having a "capitate, globose mass of gleba". Dennis (1970) repeated this information, probably based on Lloyd's description. Lloyd's belief that *Xylophallus* was a synonym of *Mutinus* has been wrongly propagated through the literature during the 20th century.

Sáenz et al. (1972) accompany their good detailed description of *X. xylogenus* from Costa Rica with an illustration of the receptacle with an umbilicate apex (FIG. 1D) and perforated apex (approximately 100 µm in diam). Baseia et al. (2003) distinctly illustrated the receptacle pore (FIG. 1E). Among the examined materials, we found specimens with rounded to umbilicate apices, minutely perforated or not. We believe that the perforated apex probably represents the mature receptacle.

Calonge et al. (2005), basing their observations on Dring (1973), cited *X. xylogenus* as cosmopolitan. However, Dring noted distribution only for the entire genus *Mutinus*, not for this species in particular. According to Gómez & Gazis (2006) and elsewhere, although *X. xylogenus* may be common in neotropical forests, the species rarely collected due to its minute size and ephemeral nature.

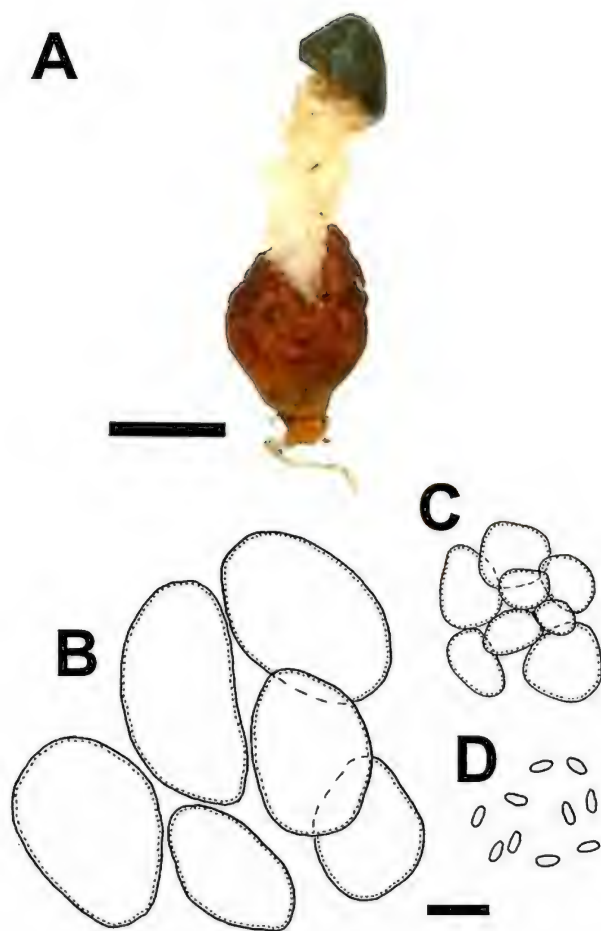


FIG. 3. *Xylophallus xylogenus*.

A. Single basidiome (scale = 2.5 mm). B–D. Microscopical structures (scale = 10 μ m):

B. Pseudoparenchymatic hyphae from the pseudostipe.

C. Pseudoparenchymatic hyphae from the volva. D. Basidiospores.

We agree with Sáenz et al. (1972) in separating *X. xylogenus* from *Mutinus*, since the gleba develops on a receptacle externally modified from the pseudostipe. Although the xylophilous habit of *X. xylogenus* is observed in other *Phallus* species, we prefer to retain the species in *Xylophallus* until further molecular analyses are carried out.

Key to small xylophilous phalloid species

- 1a. Receptacle smooth, minutely perforated; pseudostipe with rectangular alveoles (Neotropics) *Xylophallus xylogenus*
- 1b. Receptacle reticulate, clearly perforated with a conspicuous pore; pseudostipe minutely reticulate or spongy 2
- 2a. Pseudostipe yellow, basidiomata 70–100 mm high (China, Japan, Indonesia, Sri Lanka, tropical Africa) *Phallus tenuis*
- 2b. Pseudostipe white, basidiomata smaller, ≤ 45 mm high 3
- 3a. Pseudostipe spongy, 25–33 mm high; basidiospores 2–3 µm long (Tanzania, eastern Africa) *P. minusculus*
- 3b. Pseudostipe reticulate-lacunose, 20–45 mm high; basidiospores 3–3.5(–3.8) µm long (São Tomé, western Africa) *P. drewesii*

Acknowledgments

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***Plectania seaveri* (Ascomycota, Pezizales),
a new discomycete from Bermuda**MATTEO CARBONE ^{1*}, CARLO AGNELLO ² & SCOTT LAGRECA ³¹ Via Don Luigi Sturzo 173, I-16148, Genova, Italy² Via Antonio Gramsci 11, I-72023, Mesagne (BR), Italy³ Plant Pathology Herbarium, Dept. of Plant Pathology & Plant Microbe Biology,
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ABSTRACT— *Plectania seaveri* sp. nov. is described after revision of Bermudian material identified by Seaver under the name *Bulgaria melastoma*. The only known host for this new species is the endemic Bermudian tree *Juniperus bermudiana* (Bermuda cedar), a species whose numbers have greatly reduced over the past 70 years due to the introduction of invasive scale insects. As this new fungus has not been collected since 1940, it may now be extinct. Color pictures of fresh and dried specimens, microscopic morphology, and a black and white drawing of hymenial elements are included. Related, excluded, and doubtful species of *Plectania* are also discussed.

KEY WORDS— ascomycetes, *Donadinia*, taxonomy

Introduction

When the senior authors (MC, CA) began their revision of the genus *Plectania* Fuckel, they realized that the Bermudian collections reported by Seaver (1928, 1942) and Seaver & Waterston (1946) under the name *Bulgaria melastoma* [i.e., *Plectania melastoma* (Sowerby) Fuckel] required further study. After publication of Carbone et al. (2012) and Agnello & Carbone (2012), the need to re-examine these collections was even clearer. These early studies strongly indicated that Seaver's Bermudian collections might represent a new taxon, and so, joined by the third author (SLG), we examined all Bermudian material of this taxon in the Plant Pathology Herbarium of Cornell University (CUP).

Our revision rapidly confirmed that Bermudian material of *Bulgaria melastoma* in CUP does not belong to *B. melastoma* but in fact represents a strikingly different, undescribed species. We here name this species in honour



PLATE 1. F.J. Seaver in his office at NY Botanical Garden (photographed 1930–35). Photo by H.M. Fitzpatrick, from the Cornell University Plant Pathology Herbarium photograph collection.

of the original researcher for most of this material, the American mycologist Fred Jay Seaver (PLATE 1).

Materials & methods

Microscopic characters are based on dry specimens. Two optical microscopes were used: Olympus CX41 trinocular and Optika B353 trinocular with plan-achromatic objectives 4×, 10×, 40×, 60×, and 100× in oil immersion. Microscopic pictures were made using a Nikon 4500 camera and a Nikon Coolpix. Primary mounting media were Melzer's reagent, cotton blue, and Congo red. Water mounts were used for all measurements and observations of pigments. At least 30 spores were measured from each examined apothecium. SEM observations were made using a Hitachi S-4000 scanning electron microscope (SEM) at the University of Florida Core EM facility (Gainesville, Florida, USA). A piece of the hymenium from the holotype was mounted on aluminum stubs with adhesive graphite tabs and sputter coated with a gold-palladium alloy for 60 s.

Taxonomy

Plectania seaveri M. Carbone, Agnello & LaGreca, *sp. nov.*

PL. 2–6

MYCOBANK MB 563704

"*Bulgaria melastoma*" sensu Seaver, N. Amer. Cup-fung.,

Operc.: 198, 1928, Bermudian records.

Differs from *Plectania melastoma* in the absence of macroscopic orange-red warts, more stipitate apothecia, and ellipsoid and differently ornamented spores; from *P. nannfeldtii* and *P. helvelloides* in a very different spore ornamentation; and from *P. lusitanica* in larger spores and more pronounced ornamentation.

TYPE: Bermuda, Walsingham, ca. 150 fruitbodies on bark of *Juniperus bermudiana*, 20 January 1922, leg. H.H. Whetzel, Bermuda Fungi no. 188 [Holotype, CUP-Whetzel. B.F.-0188, as *Bulgaria melastoma*].

ETYMOLOGY: In honor of the American mycologist, Fred Jay Seaver.

MACROCHARACTERS (based on dried specimens) — **APOTHECIA** gregarious to subcespitose, mostly long stipitate, nearly globose and closed at first, expanding as they mature; base attached to substrate with very numerous dark black, threadlike filaments (subiculum); **CUP** ≤ 18 mm across and ≤ 6 mm high; margin mostly entire: **HYMENIUM** blackish to black, apparently smooth; **EXTERNAL SURFACE** blackish to black, with a dark brownish to purplish-amaranth shade, rough, gently wrinkled; **STIPE** ≤ 16 mm high and ≤ 2 mm thick, solid, black; the length varying depending on the point of growth from the substrate; **FLESH** leathery, darker near external surfaces.

MICROCHARACTERS — **ASCI** $350\text{--}400 \times 14\text{--}15$ μm , cylindrical, operculate, with a slightly excentric operculum, inamyloid, eight-spored, with walls ≤ 1 μm thick and a tapered, flexuous base with a generative hypha that is connected more or less distantly from the basal septum; **PARAPHYSES** not, or only slightly, exceeding the length of the asci, $1.5\text{--}2(\text{--}2.5)$ μm wide, cylindrical, septate, sometimes anastomosing, branched, pale brownish, multiguttulate by very small oil drops; the apex usually more or less diverticulate and lobed; an extracellular, brownish, amorphous pigment present in the upper part (observed in water mounts), uniting them in bundles; **SETAE** (hymenial hairs) cylindrical, as long as the paraphyses, $(3\text{--})3.5\text{--}4.5$ μm wide, with a simple to slightly subcapitate apex and single septum at the very base, pale brownish; **SPORES** ellipsoid, $22\text{--}27 \times 10\text{--}12$ μm , most frequently $23\text{--}25 \times 10\text{--}11$ μm , very few spores observed ≤ 31 μm long, $Q = (1.8\text{--})2\text{--}2.7(\text{--}3)$, subhyaline to light yellowish, filled with a granular content and sometimes with few slightly bigger oil drops, walls ≤ 1 μm thick; spore sculpturing of small but well-defined round warts ≤ 0.8 μm broad and 0.6 μm high, visible also at low magnifications in optical microscopy; confirmed by the SEM, showing round isolated warts; very young spores are round, smooth and thick walled; **SUBHYMENIUM** composed of a dense *textura intricata* of cylindrical, frequently septate, hyphae, with thickened, more or less dark brown walls; at low magnifications, it appears uniformly brownish to brown and not so sharply differentiated from the upper layer of the medullary excipulum; **MEDULLARY EXCIPULUM** of *textura intricata* with cylindrical, septate hyphae, $5\text{--}11$ μm wide, with walls on average $0.5\text{--}0.8$ μm thick; two main layers can be recognized: (i) an upper one with more brownish, denser, less gelified hyphae with a parallel orientation to the hymenial surface, (ii) a



PLATE 2. *Plectania seaveri*. Upper row: CUP-033234: fresh specimens. Photo by F.J. Seaver. Bottom row: dried specimens of holotype collection. Photos by M. Carbone. Bar = 10 mm valid for bottom row only.

middle-lower one with lighter to yellowish, more loosely interwoven hyphae immersed in a subgelatinous matrix; ECTAL EXCIPULUM of textura subglobulosa-angularis to textura angularis made up of elements $\leq 20\ \mu\text{m}$ wide and/or high, very dark brown due to the colored walls and the presence of an incrusting

brown pigment. In the very outer part the pigmentation becoming more amber-like and crystalline; EXTERNAL HAIRS mainly of two types, although intermediate forms occurring; (i) cylindrical, septate, hyaline, thin walled, heavily encrusted hyphoid hairs, $\leq 2.5 \mu\text{m}$ wide; (ii) cylindrical, infrequently septate, $\leq 6 \mu\text{m}$ wide, very long, mainly straight, smooth to very finely encrusted (only in the basal part) by the same kind of crystalline pigment, and brown due to an epimembranaceous pigmentation, with walls thickened $\leq 0.8 \mu\text{m}$; in non-squashed mounts, they are mainly lying on the external surface, and it is not easy to determine their entire length; SUBCULUM made up of cylindrical, sometimes very slightly flexuous and/or notched, $6\text{--}9 \mu\text{m}$ wide, $\leq 3 \text{ mm}$ (or more) long, septate hyphae, brown due to an epimembranaceous pigmentation, with walls thickened $\leq 0.8 \mu\text{m}$, mainly smooth but in places a very light greenish extracellular pigment is present.

ECOLOGY & DISTRIBUTION — gregarious to subcespitose, on bark of Bermuda cedar (*Juniperus bermudiana* L.). January and February. Known only from the Bermuda Archipelago.

ADDITIONAL COLLECTIONS EXAMINED: **Mature collections:** BERMUDA, Agricultural Station, eleven fruitbodies on bark of *Juniperus bermudiana* on ground, 4 February 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-033235); six fruitbodies on *J. bermudiana*, 15 January 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-034615). Burt's Island, four fruitbodies on fallen tiny twigs of *J. bermudiana*, 9 February 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-033236).

Immature collections: BERMUDA, Agricultural Station, on bark of *Juniperus bermudiana* on ground, 12 January 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-033231); on bark of living *J. bermudiana*, 28 January 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-033234). Harrington house, on fallen bark of *J. bermudiana* on ground, 23 January 1926, leg. H.H. Whetzel, Ogilvie & F.J. Seaver (CUP-033233).

Discussion

PRELIMINARY NOTES — The first hint of the existence of these Bermudian collections can be found in Seaver's note (1928) under *Bulgaria melastoma*: "What appears to be this species is very abundant in Bermuda on the bark of Bermuda cedar, *Juniperus bermudiana*, but never on anything else. Since the host is an endemic species, the fungus may also be found to differ and be itself endemic. The writer has not yet had the opportunity to clear up this point but reference will be made to it in later publications". In Seaver (1942), again under *Bulgaria melastoma*: "Range extended to Bermuda (F.J. Seaver & H.H. Whetzel)". And lastly, in Seaver & Waterston (1946): "However one of us (Seaver 1928: 198, 1942: 320) has shown that the Bermuda species is referable to *Bulgaria melastoma* (Sow.) Seaver. Figure 1 (upper) shows this species on rotten bark of *Juniperus bermudiana* L., from roots of living trees exposed at soil level, Walsingham, Bermuda, Jan. 20, 1922, H.H. Whetzel Bermuda Fungi No. 188. This plant is characterized by apothecia which are frequently stipitate,

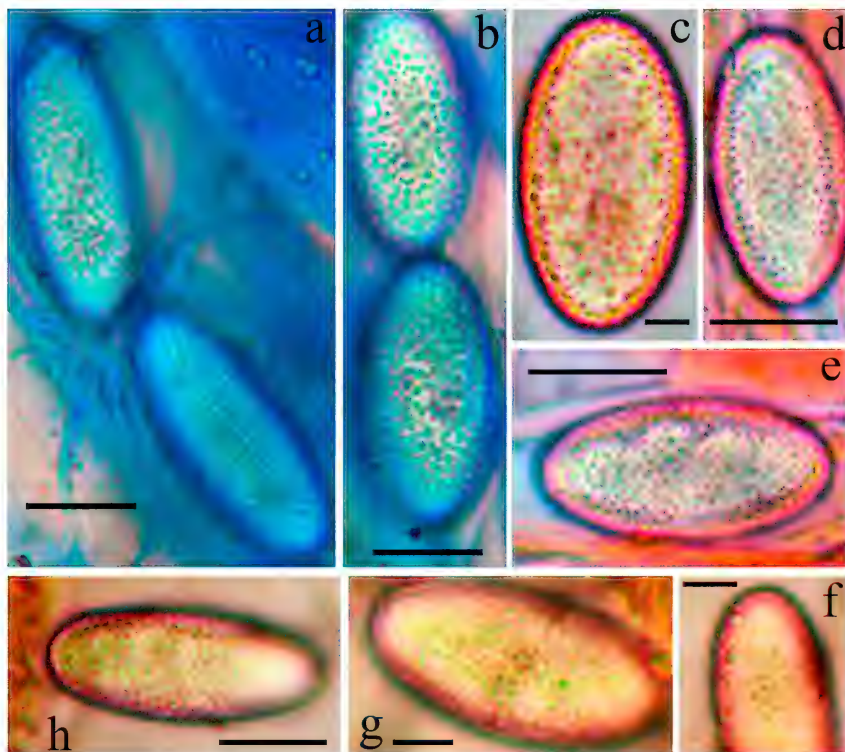


PLATE 3. *Plectania seaveri* (holotype). a–b: spores in lactic Cotton blue. c–e: spores in Congo red. f–h: spores in water mount. Bars: a, b, d, e, h = 10 µm; c = 2,5 µm; f, g = 5 µm. Photos by M. Carbone.

with almost smooth, hyaline, ellipsoidal spores, $20\text{--}25 \times 9\text{--}10\text{ }\mu$ ". In Waterston (1947) we find only a list of the collections studied here, under *Bulgaria melastoma*, and nothing is added from a taxonomic point of view.

Korf (1957) seems to have been the first mycologist to realize that these collections might represent a new taxon: "...the species of "*Bulgaria*" described by Seaver and Waterston (1946: 182) from Bermuda, which appears to be undescribed..." While revising the cited material we found that Denison (Korf's student) also noted Seaver's misinterpretation, for in collection CUP-033235, there is a handwritten annotation noting, "This is not *Bulgaria* (*Plectania*) *melastoma*. Note, for example, the delicately sculptured spores, the larger apothecia and longer stipe. W C Denison 2 Nov 1961".

Many years before, Boedijn (1932) suggested that Seaver's Bermudian *Bulgaria melastoma* (Seaver 1928) might be conspecific with *Sarcosoma thwaitesii* (Berk. & Broome) Petch [= *Galiella thwaitesii* (Berk. & Broome) Nannf.], mainly

because Petch (1910) recorded *S. thwaitesii* from Ceylon, growing on *Juniperus bermudiana*. Although Seaver & Waterston (1946) definitely misinterpreted *Bulgaria melastoma*, they demonstrated as well that Boedijn (1932) was wrong. *Galiella thwaitesii* is undoubtedly a very distinct and different taxon. For further details about *Galiella thwaitesii* see Le Gal (1953).

SPECIES DELIMITATION — *Plectania melastoma*, in its original sense, is definitely a very different species from the material studied here. As demonstrated by Agnello & Carbone (2012) the macroscopic, vivid orange-red, external warts are always present, and still visible to the naked eye even after 150 years; in addition, the apothecia are at most substipitate and never definitely stipitate as in *P. seaveri*. Microscopically, there are more numerous and immediately appreciable differences. For example, in *P. seaveri* the spore sculpturing is delicate but, at the same time, very distinct and pronounced. It is easily visible in water mounts (1000×, oil immersion). In contrast, in *P. melastoma*, the spores are far less sculptured and have very different warts that are barely visible, even in 1000× Cotton blue mounts. The spore shapes are also quite different — mostly subfusoid in *P. melastoma*, ellipsoid in *P. seaveri*. Lastly, *P. melastoma* produces only one kind of external hairs and a differently coloured crystalline pigmentation that is mostly a vivid orange-red-amber, not pale brownish to dark amber as in *P. seaveri*.

Unlike *Plectania melastoma*, *P. seaveri* surely belongs to *Plectania* sect. *Donadinia* (Bellem. & Mel.-Howell) M. Carbone & Agnello. In this section, Carbone et al. (2012) recognize only three stipitate species with more or less defined ornamented ellipsoid spores: *P. helvelloides* (Donadini et al.) Donadini, *P. nannfeldtii* Korf, and *P. lusitanica* (Torrend & Boud.) M. Carbone et al. As pointed out by Carbone et al. (2012), while all these species have extremely similar gross macroscopic morphologies, excipular anatomies, and external hairs, they can clearly be distinguished based on the combination of spore size and spore sculpturing. In fact, if on one hand we observe that spore measurements show a continuum from a minimum of 16 µm (in *Plectania lusitanica*) to a maximum of 38 µm (in *Plectania nannfeldtii*), on the other hand we are presently unaware of any populations that show a simultaneous overlap of spore size range and sculpturing. SEM and optical observations also support this morphological distinction.

The well-known American “snowbank mushroom,” *Plectania nannfeldtii*, can be immediately differentiated by its different ecology, geographical distribution, longer spores, and different episporium (e.g. Seaver 1928; Miller 1967; Smith et al. 1981; Arora 1986; Tylutki 1993; Li & Kimbrough 1995). *Plectania lusitanica* surely shares a similar ornamentation but differs in its smaller spores and a much less pronounced spore sculpturing that is barely visible in optical microscopy (data taken from many spore prints). Lastly, *P. helvelloides* spores

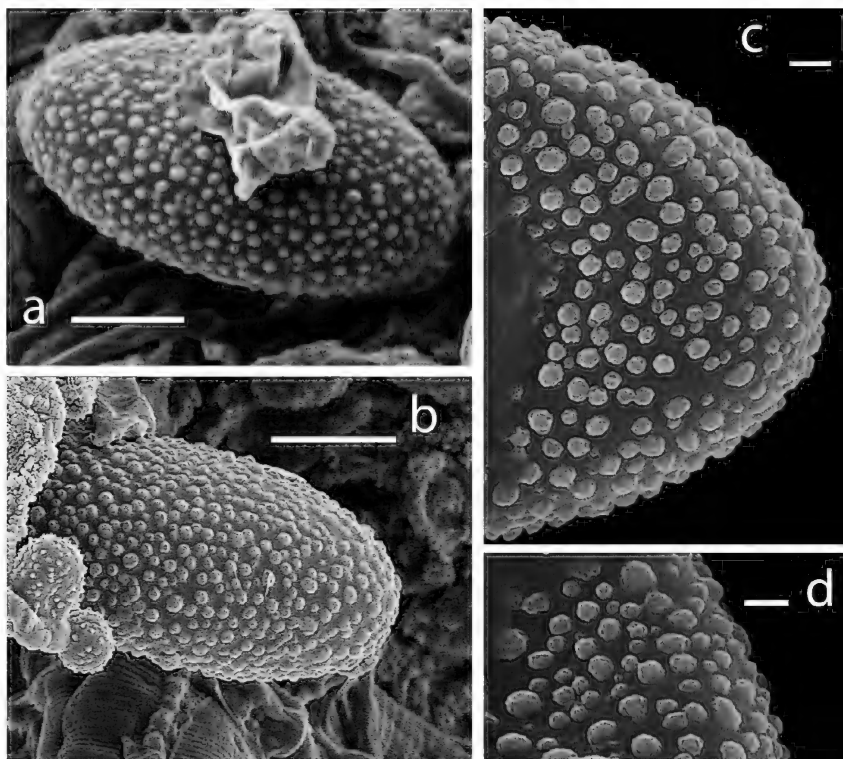


PLATE 4. *Plectania seaveri* (holotype). a–b: spores. c: pole of the spore. d: spore sculpturing. Bars a–b = 5 µm; c–d = 1 µm. SEM courtesy of Mark Whitten and the Electron Microscopy Core Facility at the University of Florida (Gainesville, USA).

(22–28 × 9–13 µm; Donadini et al. 1973, Carbone et al. 2012), which might be regarded as the closest to *P. seaveri* in size, have a different ornamentation type — relatively easy to see in cotton blue (1000×), but smaller, differently shaped, and not easily detectable in outline.

The spore sculpturing seen in *Plectania seaveri* is extremely similar to that found in *Galiella* Nannf. & Korf, which, however, produces very different fleshy large gelatinous apothecia; for a quick survey, see Boedijn (1932, as *Sarcosoma*), Le Gal (1953, as *Sarcosoma*), Korf (1957), Cao et al. (1992), and Zhuang & Wang (1998). The same can be said for the genus *Neournula* Paden & Tylutki, previously placed in *Sarcosomataceae* Kobayasi but now transferred into a different family, *Chorioactidaceae* Pfister. *Neournula* does indeed possess similarly warted spores but is distinct from *Plectania* in many other features (see Pfister et al. 2008).

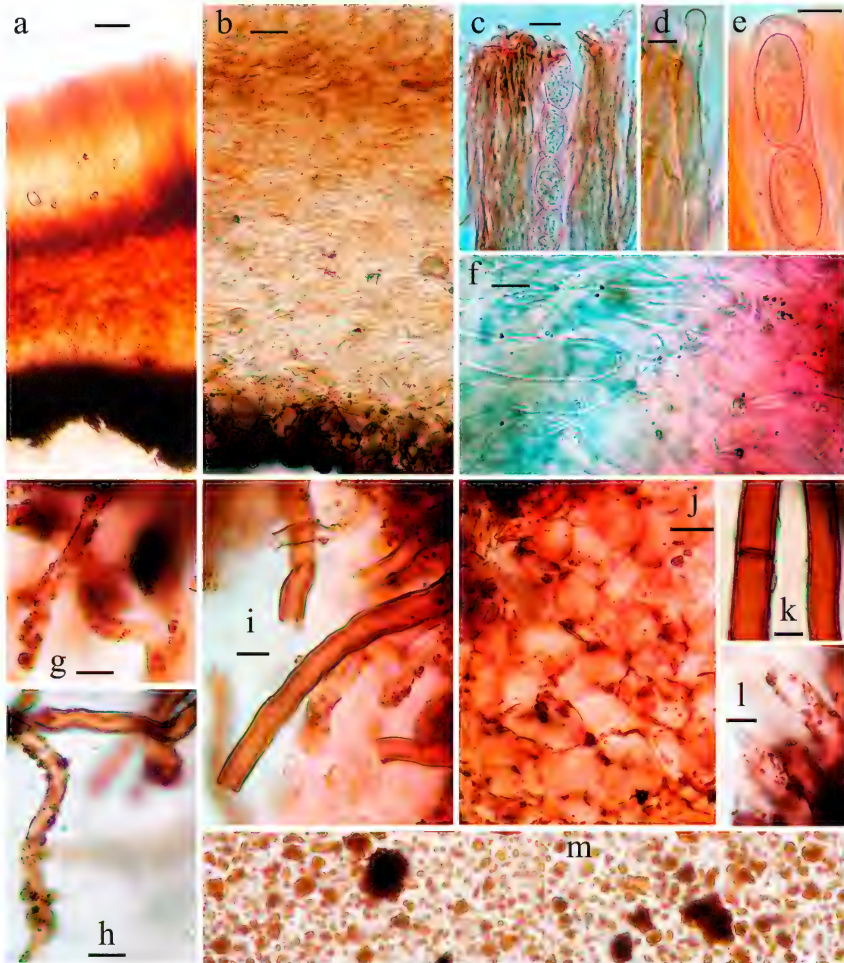


PLATE 5. *Plectania seaveri* (holotype). a: section of the apothecium; b: medullary excipulum in water mount; c: bundles of paraphyses; d: seta (hymenial hair); e: ascus tip; f: medullary excipulum in Congo red (magnified); g–h: hyphoid excipular hairs; i: excipular hairs; j: ectal excipulum; k: subiculum; l: encrusted cells of the margin; m: crystals of excipular pigment. Bars: a = 100 μ m; b = 20 μ m; c, e, f, j = 10 μ m; d, g, h, i, k, l = 5 μ m. Photos by M. Carbone.

EXTRALIMITAL AND DOUBTFUL SPECIES — Mention must be made here of two other *Plectania* species described from the Americas. *Plectania coelopus* (Mont.) Sacc. was described from Chile and, according to the iconotype and original description, it surely belongs to sect. *Donadinia*. We agree with Le Gal (1958) and Cabello (1988) that *P. coelopus* must be regarded as a doubtful species until

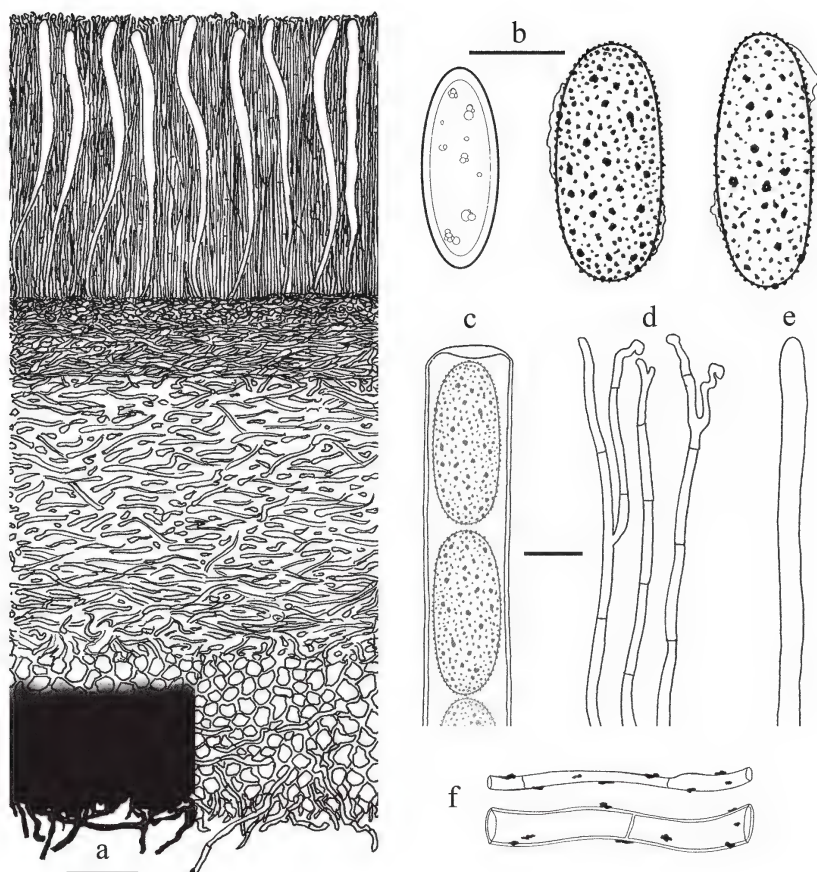


PLATE 6. *Plectania seaveri* (holotype). a: section of the cup; b: spores; c: ascus; d: paraphyses; e: seta (hymenial hair); f: excipular hairs. Bars: a = 100 μ m; b = 10 μ m; c–f = 10 μ m. Drawing by C. Agnello.

its real identity is investigated and/or it is typified with topotypical (or other Chilean) material.

Another doubtful American taxon is *Plectania rimosa* Peck, described from California (Peck 1903). Seaver (1942) treated it as a “doubtful species”, and due to the (apparent) lack of original material, he stated that this species might represent *Paxina corium* (O. Weberb.) Seaver [= *Helvella corium* (O. Weberb.) Masee]. As the protologue does not fit *Helvella corium*, we disagree and regard *Plectania rimosa* as a nomen dubium.

During our literature searches, we have found other unclear species seemingly in need of revision (e.g., *Neourmula helvelloides* sensu Zhuang & Wang (1998) and *Plectania nannfeldtii* in the sense of the Chinese authors Teng (1963), Tai (1979), and Bi et al. (1990) and the Japanese author Otani (1973). Future studies will address the misapplications of these names.

ECOLOGY — All known *Plectania seaveri* specimens were collected from rotting bark of *Juniperus bermudiana*, an endemic Bermudian tree that historically dominated the upland forests of the Bermuda Archipelago (Thomas 2004). Most collections of this fungus were made in the 1920s, with the last known specimen collected in 1940 (Waterston, 1947). A search of 125 fungus specimens from the last major Bermudian mycological expedition collected by Richard Korf and colleagues during the week of January 17–23, 1980 (unpublished; specimens deposited at CUP) revealed no additional *P. seaveri* specimens.

Between 1946 and 1953, introduced scale insects decimated the Bermuda cedar, killing an estimated 95% (Phillips 1984), thus deforesting the islands and altering the ecology of many other Bermudian species in major ways (Thomas 2004). With no known *Plectania seaveri* specimens collected since 1940, it seems probable that this fungus is now extinct. While it may still occur on some of the dead, non-decorticated trunks of Bermuda cedar that now dot the Bermudian landscape (and perhaps on the few living Bermuda cedars which still persist), it seems likely that the fate of *P. seaveri* may eventually follow that of other endemic Bermudian organisms dependent on Bermuda cedar, such as the Bermuda cicada (*Tibicen bermudiana*) and the ochre-banded looper moth (*Semiothisa ochrifascia*) (Sterrer 1998).

As far as we know, all species of *Plectania* sect. *Donadinia*, including *P. seaveri*, associate as saprophytes with coniferous trees. Future collections of representatives of this section would help us understand whether this coniferous association is taxonomically relevant.

FINAL CONSIDERATIONS — This study demonstrates that *Bulgaria melastoma* as interpreted by Seaver (1928) needs an in-depth revision. Paden & Tylutki (1969) observed that “in Seaver, *P. milleri* will key out to *P. melastoma* (as *Bulgaria melastoma*).” We can speculate that, at least in the case of *Plectania seaveri*, Seaver might have misinterpreted the encrusting pigment of the external surface, indicating that he was not aware of the true diagnostic characters of *P. melastoma*. In any case, we are convinced that Seaver misapplied the name *P. melastoma* to other specimens, such as one from Puerto Rico (CUP-PR-001281, examined as part of the current study), and probably also the Jamaican collection cited by Seaver & Waterston (1946). The latter specimen, supposedly deposited in NY, could not be located during a recent visit to that herbarium (SLG pers. obs., April 2, 2012). Our future studies will attempt to answer these lingering questions.

Acknowledgments

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**New species of *Entoloma* (Basidiomycetes, Agaricales)
from Kerala State, India**C.K. PRADEEP^{1*}, K.B. VRINDA¹, SHIBU P. VARGHESE¹ & T.J. BARONI²¹Tropical Botanic Garden & Research Institute,
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ABSTRACT — Three new species of *Entoloma* (*E. suaveolens*, *E. crassum*, and *E. aurantio-quadratum*) are described, illustrated, and discussed based on collections made from Kerala State, India.

KEY WORDS — agaric diversity, under-explored, Western Ghats

Introduction

Entoloma is one of the largest genera of *Agaricales*, with more than 1500 species known worldwide (Noordeloos 1981a). However, vast areas are still under-explored, especially in Africa, South America, India, and South East Asia (Noordeloos & Morozova 2010). Recent studies of the genus in Kerala State, India, by Manimohan et al. (1995, 2002, 2006) documented 39 species, of which 26 are new to science, indicating that *Entoloma* is especially rich in the state. While documenting the agaric diversity of Western Ghats of Kerala, the authors collected several specimens of *Entoloma*. Our detailed study of these collections has resulted in three new species, which are described, illustrated, and discussed here.

Materials & methods

Gross morphological descriptions are based exclusively on fresh materials collected from Kerala State, India. Color coding follows Kornerup & Wanscher (1978). Microscopic characters were studied from hand cut sections of dried basidiomata revived in a 3% KOH aqueous solution, stained with 1% Congo red, and examined under a Leica DME 1000 compound microscope. The mean quotient (Q) of spore length divided by spore width was calculated from measurements of 30 basidiospores; the hilar

appendix was included in the measurement of the spore length. Line drawings were made with assistance of an attached drawing tube. All specimens cited were collected by the authors unless otherwise indicated. Holotypes are deposited at the Herbarium of the Royal Botanic Gardens, Kew (K), and all isotypes and additional materials examined are deposited at the Mycological Herbarium of Tropical Botanic Garden and Research Institute, Trivandrum (TBGT).

Taxonomy

Entoloma suaveolens C.K. Pradeep & K.B. Vrinda, **sp. nov.** PLATE 1A–B; FIG. 1

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Differs from other blue *Entoloma* species in combining an appressed squamulose pileus and stipe with the lack of hymenial cystidia and the presence of a strong fragrant odor.

TYPE – India, Kerala State, Trivandrum District, Palode, TBGRI campus, 25 Sep 1996, Pradeep 3621 (**Holotype**, K(M) 172393).

ETYMOLOGY –*suaveolens* refers to the fragrant odor of the basidiomes

PILEUS 20–53 mm diam., convex with a broad obtuse umbo; surface ink blue, dark blue to greyish blue (20F4/21F4/21F8/22F4/21D5) at disc with greyish violet to dark blue (18E5/19E4/19E5/19E6) elsewhere, appressed squamulose throughout, often cracked to expose the white underlying context in dry season; squamules often washed off during heavy rain towards margin to appear subsquamulose, dry; margin straight, entire. LAMELLAE adnate, white, cream to greyish orange (4A3/4B4/5A2/5B3/6A2/6B4), ≤ 11 mm wide, close to crowded, with lamellulae of different lengths; edge concolorous, entire. STIPE 35–114 × 3–10 mm, central, cylindric, curved, fistulose to narrowly hollow, brittle, tapering up from a subclavate base; surface mostly concolorous with pileus or greyish violet (19D5), greyish blue (20D5), appressed squamulose throughout; base white with white mycelial mat and mycelial cords. CONTEXT white, up to 3 mm, soft. ODOR strong, distinct, pleasant, fragrant, similar to holy ash. SPORE PRINT pink.

BASIDIOSPORES 10–12 × 5–7.5(–8) µm, avL = 11, avW = 6.77, Q = 1.3–1.7, avQ = 1.65, heterodiametric ovate, 5–7 facets in profile. BASIDIA 32–42.5 × 8.5–14 µm, clavate, 4-spored; sterigmata up to 4.5 µm long. LAMELLA EDGE fertile. CHEILOCYSTIDIA and pleurocystidia absent. HYMENOPHORAL TRAMA regular; hyphae 2.5–16(–28) µm wide, thin-walled, hyaline. PILEAL TRAMA interwoven; hyphae similar to hymenophoral trama. PILEIPELLIS a trichoderm with clavate to cylindro-clavate elements, 62.5–147 × 10–14 µm, thin-walled with bluish (in water) contents which dissolve in 3% KOH. STIPITEPELLIS a cutis of loosely arranged septate thin-walled hyphae, 2.4–4.8 µm wide, with pale greyish plasmatic contents. CAULOCYSTIDIA in tufts present on the upper part of the stipe, 41–67 × 5–9 µm, cylindro-clavate, thin-walled with pale brown vacuolar contents. CLAMP CONNECTIONS and oleiferous hyphae present in all parts.

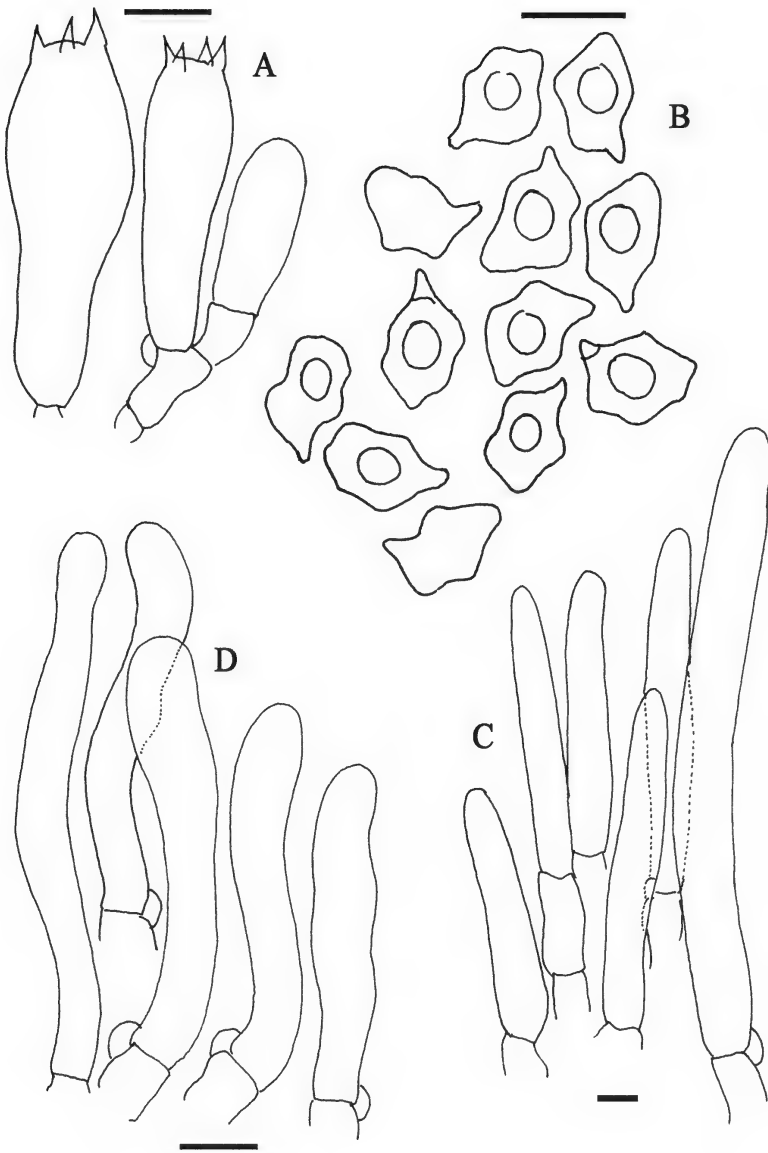


FIG. 1: *Entoloma suaveolens*. A, basidia; B, basidiospores; C, trichodermial elements; D, caulocystidia. Scale bars = 10 μ m.

HABIT & HABITAT — Solitary, scattered on soil among decayed litter in evergreen forest, June–December.

ADDITIONAL SPECIMENS EXAMINED — **INDIA, KERALA STATE**, TRIVANDRUM DISTRICT, Palode, TBGRI campus: 16 Sep 1997, TBGT 3887; 15 Oct 2001, TBGT 6770; 8 Jun 2005, TBGT 8907; 4 Nov 2005, TBGT 9425; 17 Jul 2007, TBGT 10427; 25 Jul 2008, TBGT 11513; 30 Jul 2008, TBGT 11554; 16 Dec 2009, TBGT 13058; **WAYANAD DISTRICT**, Nadavayal: 27 Sep 2007, TBGT 10575 (all at TBGT).

DISCUSSION — *Entoloma suaveolens* is characterized by the unique combination of medium to large bluish terrestrial tricholomatoid basidiomes, convex umbonate appressed squamulose pileus and stipe, adnate pink lamellae, heterodiametric ovate basidiospores, absence of hymenial cystidia, trichodermial pileipellis, abundant caulocystidia, clamp connections present in all hyphae, and strong fragrant odor.

The present collections invite comparison with a number of blue *Entoloma* species described from various regions of the world by different workers. *Entoloma rugosopruinatum* Corner & E. Horak, originally described from Sabah (Horak 1980) and recently recorded from Kerala (Manimohan et al. 1995), differs by its violaceous purple finely tomentose pileus, presence of cylindro-clavate cheilocystidia, and subfarinaceous odor. *Entoloma divum* Corner & E. Horak is distinguished by a deep blue pruinose pileus, palisadic pileipellis, and lack of any odor. Three other comparable blue species — *E. simillimum* Corner & E. Horak, *E. egregium* E. Horak, *E. marinum* Corner & E. Horak — are easily separated by their velutinous pileus, presence of cheilocystidia, and lack of any distinctive odor. The European taxa *E. chalybeum* (Pers.) Noordel. and *E. chalybeum* var. *lazulinum* (Fr.) Noordel. (Noordeloos 1992) are distinguished by their depressed velutinous pileus, heterodiametrical 5–9-angled spores, sterile lamella edge, a cutis pileipellis, and absence of clamps. *Entoloma bloxamii* (Berk. & Broome) Sacc. and *E. nitidum* Quél. (Horak 1973, 1978, 1980; Noordeloos 1992), though comparable in gross morphology and pigmentation, vary markedly in their smooth glabrous subviscid pileus (pileipellis an ixocutis) and distinctive farinaceous odor. *Entoloma dichroum* (Pers.) P. Kumm. (Noordeloos 1992) differs in its violaceous purple pileus, heterogeneous lamella edge, and unpleasant odor. The recently described *E. eugenei* Noordel. & O. Morozova (Noordeloos & Morozova 2010) from Russia differs in its velvety deep blue pileus, sterile lamellae edge with cylindrical, narrowly lageniform or irregularly shaped cheilocystidia, and mild spicy odor.

Leptonia carnea Largent, a rather uncommon species restricted to coastal and northern California, is characterized by a blue black densely appressed fibrillose squamulose pileus and stipe, blue–violet–black lamella edge, cuticular pileipellis, and distinctive farinaceous odor and taste (Largent 1977); *L. carnea* is further distinguished by the insoluble blue pigment, contrasting with the readily KOH-soluble pigment in *E. suaveolens*.

Two recently described blue species from Kerala State, *E. griseolazulinum* Manim. & Noordel. and *E. indoviolaceum* Manim. & Noordel. (Manimohan et al 2006), clearly differ in their velutinous pileus, presence of cheilocystidia, large heterodiametric spores, and lack of any distinct odor.

Tricholomatoid habit, convex umbonate bluish pileus, trichodermial pileipellis, and presence of clamp connections place this species in subg. *Leptonia* sect. *Leptonia* (Noordeloos 1981a).

***Entoloma crassum* C.K. Pradeep & K.B. Vrinda, sp. nov.**

PLATE 1C–D; FIG. 2

MYCOBANK MB 564455

Differs from *Entoloma flavidum* in its glabrous creamy to yellow white pileus, lack of cheilocystidia, and strong, nauseating odor.

TYPE – India, Kerala State, Trivandrum District, Palode, TBGRI campus: 7 Oct 2004, Pradeep 8154 (Holotype, K(M) 172394).

ETYMOLOGY – *crassum* refers to the thick, stout basidiomes

BASIDIOMES thick, fleshy, robust, tricholomatoid. PILEUS 45–160 mm diam., convex to planoconvex with a broad obtuse umbo, becoming uplifted in old ones; surface uniformly creamy white to yellowish white (4A2/4A3) when young and yellowish white to brownish orange (4A2/4A3/3B3/3C3/4B2/ 4B3/4B4) with brownish disc (4B3/4B4/4C4/5C2/5C3/5D3/5D4/5E3/5E4/6E4) in mature ones, moist, smooth, glabrous, not hygrophanous; margin incurved when young becoming straight, wavy, folded, entire to incised. LAMELLAE broadly adnate to subdecurrent, yellowish white to greyish red (4A2/5A2/6A2/5B3/6C6/7B4), ≤ 20 mm wide, close to crowded with lamellulae of 3–4 lengths; edge concolourous, entire. STIPE 35–150 × 10–25 mm, central (often excentric in some specimens), cylindric (rarely compressed), twisted, thick, fibrous, solid, equal, with a slightly broad base; surface white, cream, ivory (4B3) turning brownish orange (5C3), hair brown (5E4), greyish brown (6D3), brownish grey (6E2) on handling, fibrillose striate, smooth. Basal white mycelial mat present in some specimens. CONTEXT white, ≤ 11 mm thick, soft, fleshy. ODOR strong, unpleasant, pungent, nauseating. SPORE PRINT cinnamon to reddish brown (6D6/8D6).

BASIDIOSPORES (8–)9–10 × (6–)6.5–7.5 μm, avL = 9.3, avW = 6.8, Q = 1.25–1.50, avQ = 1.34, heterodiametric ovate with 5–6 facets in profile. Basidia 45–58.5 × 7.5–10 μm, clavate, 4-spored. LAMELLA EDGE fertile, CHEILOCYSTIDIA and pleurocystidia absent. HYMENOPHORAL TRAMA regular; hyphae 2.5–14 μm wide, thin-walled, hyaline. PILEAL TRAMA interwoven; hyphae similar to that of hymenophoral trama. PILEIPELLIS a cutis passing to a trichoderm at the centre, elements 15–38 × 4–8 μm, clavate, cylindro-clavate to strangulated, thin-walled, hyaline. STIPITPELLIS a cutis of longitudinally parallel hyphae, 2.5–5 μm wide, thin-walled, hyaline. CAULOCYSTIDIA abundant throughout the stipe surface, 25–80 × 6–9 μm, lageniform or narrowly utriform, cylindric, cylindro-

clavate, subutriform, strangulated, flexuous, often with a short subobtuse apex, thin-walled with intracellular granular contents. CLAMP CONNECTIONS and OLEIFEROUS HYPHAE present.

HABIT & HABITAT — Solitary, scattered or in groups on soil among litter in evergreen forest or under reeds in sandy soil on riverbanks, April–December.

ADDITIONAL SPECIMENS EXAMINED — **INDIA, KERALA STATE, TRIVANDRUM DISTRICT,** Palode, TBGRI campus: 21 Oct 1995, TBGT 2758; 19 May 1997, TBGT 3858; 13 May 1999, TBGT 4659; 19 Jun 2000, TBGT 5072; 21 Jun 2000, TBGT 5081; 27 Jun 2001, TBGT 5351; 29 Apr 2002, TBGT 5502; 7 Jul 2005, TBGT 9064; 23 Sep 2005, TBGT 9278; 17 Jun 2008, TBGT 11122; 25 Jun 2008, TBGT 11237; 30 Sep 2008, TBGT 12064; 4 Nov 2008, TBGT 12189; 11 Jun 2009, TBGT 12577; 16 Jun 2009, TBGT 12606; 29 Jun 2009, TBGT 12685; 16 Sep 2009, TBGT 12918; 23 Sep 2009, TBGT 12940; 14 Oct 2009, TBGT 12980; 23 Oct 2009, TBGT 13012; 25 Nov 2009, TBGT 13110; 11 Dec 2009, TBGT 13128; 6 Jul 2010, TBGT 13373; 4 Aug 2011, TBGT 13768; Kallar: 10 Jul 2007, TBGT 10411 (all at TBGT).

DISCUSSION — *Entoloma crassum* is a very remarkable species characterized by large robust tricholomatoid basidiomata, a convex umbonate smooth glabrous yellowish white pileus with a brownish disc, broadly adnate to subdecurrent lamellae, a fibrillose striate white stipe that bruises brown, strong unpleasant nauseating odor, heterodiametric spores, fertile lamella edge, trichodermial pileipellis, caulocystidia that are abundant over the entire stipe surface, and presence of clamp connections in all hyphae.

The most closely related entolomas with large tricholomatoid yellowish white basidiomes are compared and discussed here. Despite its macroscopic and microscopic similarities, *E. flavidum* (Masse) Corner & E. Horak (Horak 1980) differs from *E. crassum* in its white subtomentose to floccose pileus that yellows with age, hollow fibrillose-striate non-yellowing white stipe, cylindric to subclavate cheilocystidia arrayed on a sterile lamella edge, a cuticular pileipellis, sour odor, and absence of caulocystidia. Among similar species that differ by possessing an ixocuticular pileipellis, *E. sinuatum* (Bull.) P. Kumm. (Noordeloos 1981b) is further distinguished by its viscid pale ochraceous pileus, characteristically yellow lamellae, and small isodiametric spores; *E. niphoides* Romagn. ex Noordel. (Largent 1994) is separated by its snow-white pileus and stipe and fruity odor; *E. moserianum* Noordel. (Noordeloos 1983) is distinguished by its typically yellow spotted pileus, lamellae, and stipe and sterile lamella edge; *E. albomagnum* G.M. Gates & Noordel. and *E. cretaceum* G.M. Gates & Noordel. (Gates & Noordeloos 2007) disagree by their pure white pileus and small spores; and *E. sepium* (Noulet & Dass.) Richon & Roze and *E. saundersii* (Fr.) Sacc. (Breitenbach & Kränzlin 1995) differ mainly in their silky white pileus, farinaceous odor, and lack of caulocystidia.

The large tricholomatoid habit, nonhygrophanous convex umbonate smooth pileus, adnate decurrent lamellae, regular hymenophoral trama, and abundant

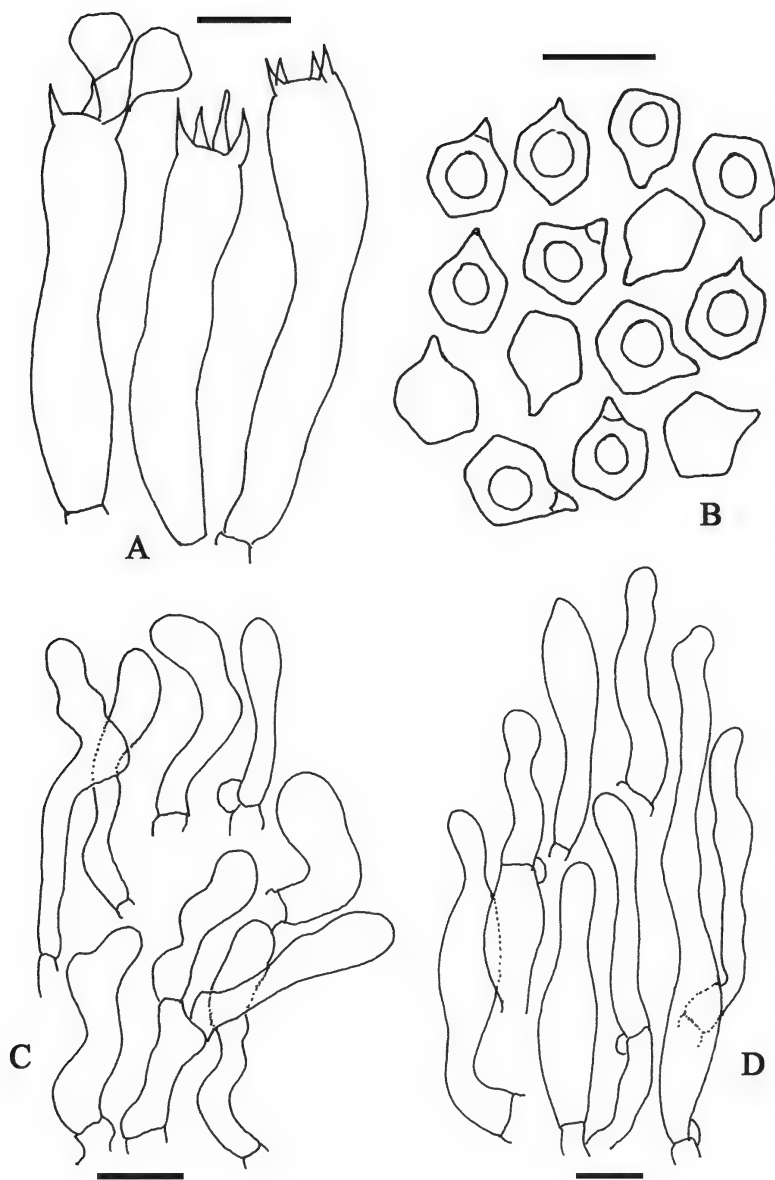


FIG. 2: *Entoloma crassum*.

A, basidia; B, basidiospores; C, trichodermial elements; D, caulocystidia. Scale bars = 10 μ m.

clamps indicate that the species belongs to subg. *Entoloma* sect. *Entoloma* (Noordeloos 1981a).

***Entoloma aurantioquadratum* C.K. Pradeep & K.B. Vrinda, sp. nov.**

MYCOBANK MB 564456

PLATE 1E–F; FIG. 3

Differs from *Entoloma talisporum* and *E. gracilius* in the absence of clamp connections, from *E. hyalodepas* in its large quadrate basidiospores, and from *E. albogracile* in the absence of hymenial cystidia.

TYPE – India, Kerala State, Trivandrum District, Palode, TBGRI campus: 11 Nov 2009, Shibu 13048 (Holotype, K(M) 172395).

ETYMOLOGY — *aurantioquadratum* refers to the orange basidiome with quadrate spores

PILEUS 10–30 mm diam., convex to applanate with a small shallow depression at centre, often uplifted in old ones; surface orange white to brownish orange (5A2/5A3/5B2/5B3/5C3), with hair brown to greyish brown (5E4/7D3/7E3) centre or even paler towards margin, dry, velvety at disc (under a lens), elsewhere smooth, glabrous, nonhygrophanous; margin straight to uplifted in old ones, wavy, entire to incised, pellucid striate when wet. LAMELLAE adnexed, orange white to pinkish white (5A2/7A2), up to 4 mm wide, close with lamellulae of different lengths; edge concolourous to the sides, entire. STIPE 20–50 × 1–3 mm, central, cylindric, twisted, hollow, equal, curved, often narrowly tapering up from a slightly broad base; surface white turning brown on handling or bruising, pruinose in the upper part (under the lens) elsewhere smooth and glabrous. CONTEXT thin, concolourous to pileus, soft. Odor none.

BASIDIOSPORES (9–)9.5–12.5(–13) × (6–)6.5–8.5(–9.5) µm, avL = 10.8, avW = 7.6, Q = 1.05–1.7, avQ = 1.4, quadrate in profile. Basidia 23–36 × 9–12.5 µm, clavate, 4-spored. LAMELLA EDGE fertile, CHEILOCYSTIDIA and pleurocystidia absent. HYMENOPHORAL TRAMA regular, 2–12 µm wide, thin-walled, hyaline. PILEAL TRAMA interwoven; hyphae similar to hymenophoral trama. PILEIPELLIS a cutis with transitions towards a trichoderm at centre, elements 19.5–65 × 7.5–11 µm, clavate to cylindro-clavate, thin-walled with brown plasmatic intracellular contents. STIPITPELLIS a cutis of loosely arranged, septate, thin-walled, 2–4 µm wide, hyaline hyphae. CAULOCYSTIDIA in groups on the upper part, 14–32 × 4.5–10.5 µm, clavate, cylindro-clavate with septate base, thin-walled, hyaline. CLAMP CONNECTIONS absent. OLEIFEROUS HYPHAE present.

HABIT & HABITAT — Scattered on forest floor in tropical evergreen forest, July–November.

ADDITIONAL SPECIMENS EXAMINED — INDIA, KERALA STATE, TRIVANDRUM DISTRICT, TBGRI campus: 10 Nov 2009, TBGT 13043; 15 Jul 2011, TBGT 13685; 11 Aug 2011, TBGT 13781 (all at TBGT).

DISCUSSION — *Entoloma aurantioquadratum* is a typical member of subg. *Inocephalus* due to its nonhygrophanous nonstriate pileus and quadrate spores.

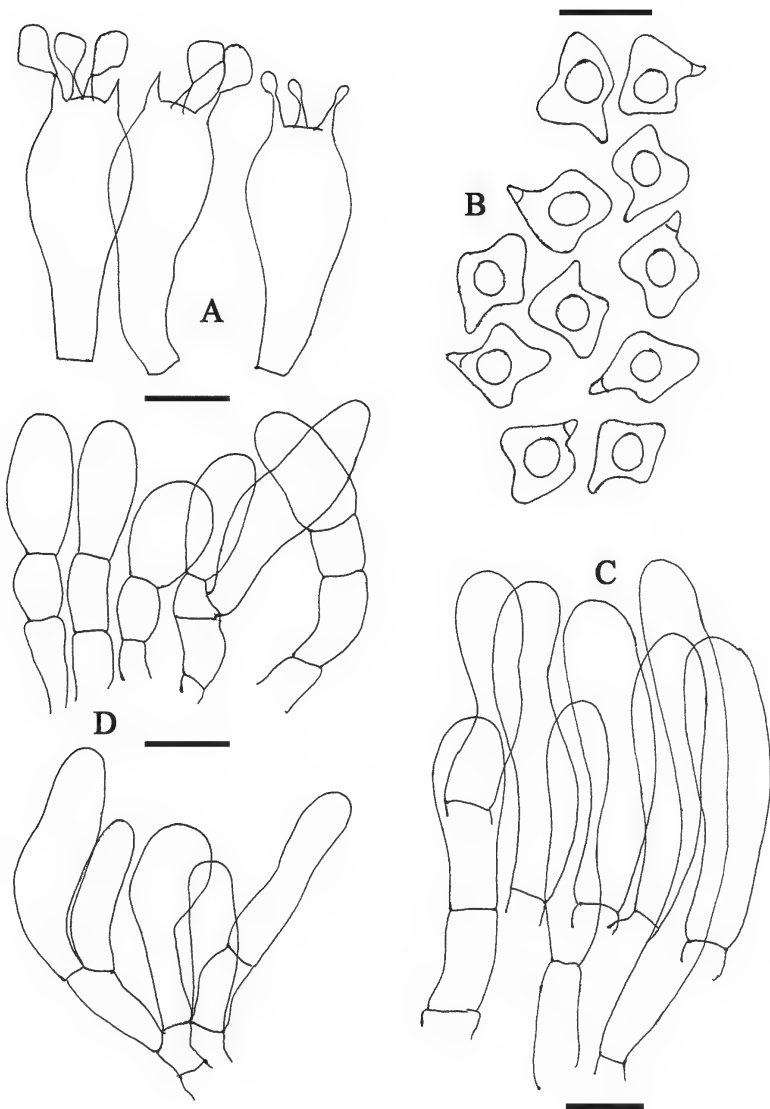


FIG. 3: *Entoloma aurantioquadratum*.

A, basidia; B, basidiospores; C, trichodermial elements; D, caulocystidia. Scale bars = 10 μ m.



PLATE 1: A–B, *Entoloma suaveolens*; C–D, *E. crassum*;
E–F, *E. aurantioquadratum*. Scale bars: A–B, E–F = 10 mm; C–D = 20 mm.

The combination of small collybioid basidiomes with pale orange pileus, white stipe turning brownish, large spores, fertile lamellae edge, cuticular pileipellis with transitions towards a trichoderm, presence of caulocystidia, and lack of clamp connections are diagnostic for this species.

A comprehensive literature search (Horak 1976, 1977, Hesler 1967, Pegler 1986, Noordeloos 1987, 1992, Noordeloos & Hausknecht 2007, Manimohan et al. 1995, 2006) revealed that although some species are comparable, none matches *E. aurantioquadratum* exactly. *Entoloma talisporum* Corner & E. Horak differs in its yellowing white fibrillose pileus, smaller cuboid spores, and presence of clamp connections. *Entoloma hyalodepas* (Berk. & Broome) E. Horak is distinguished by its depressed to infundibuliform ivory yellow or ochraceous buff pileus, decurrent lamellae, large pentagonal spores, and cuticular pileipellis (Baroni & Lodge 1998). *Entoloma albogracile* E. Horak has a convex-umbonate papillate white to yellowish pileus, cylindrical to subclavate cheilocystidia, and cuboid to tetrahedral spores. *Entoloma gracilius* E. Horak can be separated by its small fragile basidiomes, yellowish green tinted pileus, broadly adnate lamellae, smaller cuboid spores, and presence of clamp connections.

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A new species of *Infundichalara* from pine litter

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ABSTRACT — A new species of *Infundichalara* (anamorphic *Helotiales*), a hitherto monotypic genus, is described from pine needle litter. *Infundichalara minuta* sp. nov. forms two conidiophore types. Erect penicillate conidiophores producing catenulate non-septate hyaline conidia form more frequently than *Chalara*-like conidiophores with funnel-shaped collarettes produce wedge-shaped phialoconidia. Although its morphological characters correspond more with *Xenopolyscytalum*, a three-region DNA analyses (ITS, 28S rDNA and EF-1 α) of *Infundichalara*, *Xenopolyscytalum*, and related *Chalara* species in the *Helotiales* confirm the phylogenetic position of the new species within *Infundichalara*. It differs from *I. microchona* by smaller phialides and wedge-shaped conidia.

KEY WORDS — hyphomycete, dimorphic conidiophores, litter saprotroph

Introduction

Scots pine (*Pinus sylvestris* L.) needles in litter host several microfungal species producing straight erect penicillate conidiophores with catenulate hyaline conidia. *Polyscytalum fecundissimum* Riess and *P. pini* P.M. Kirk & Minter produce uniseptate conidia from denticulate conidiogenous cells growing on a straight, erect conidiophore (Kirk 1983). *Hormiactella asetosa* Hol.-Jech., differing from *Polyscytalum* by conidia with rounded ends and dark grey colonies (Holubová-Jechová 1978), predominantly colonizes bark but may also be found on needles in the litter (Koukol 2007). A monotypic genus *Xenopolyscytalum* Crous was described for *X. pinea* Crous to accommodate two strains isolated from pine needles (Crous & Groenewald 2010). Unlike *Polyscytalum* and *Hormiactella* representatives, *X. pinea* forms white tufts on needles and is characterized by aseptate conidia with somewhat darkened hila and a *Chalara*-like synanamorph. The synanamorph produces phialides with a flaring collarette that differs from the tubular collarette found in *Chalara* (Corda)

Rabenh. (Nag Raj & Kendrick 1975). The only *Chalara* species characterized by a funnel-shaped collarette, *C. microchona* W. Gams, was recently placed into a new genus, *Infundichalara* Réblová & W. Gams (Réblová et al. 2011).

I have isolated one fungus with dimorphic (penicillate/*Chalara*-like) conidiophores from needles of several pine species from various parts of Europe. These isolates are morphologically similar to both *Xenopolyscytalum* (abundant production of penicillate conidiophores) and *Infundichalara* (*Chalara*-like conidiophores with funnel-shaped collarettes) but obviously represent a distinct species. Both morphological and molecular criteria were considered to accommodate the species in a genus reflecting evolutionary relationships.

Materials & methods

Collection and isolation

Cultures were isolated from needles of Scots pine (*Pinus sylvestris*), Swiss stone pine (*P. cembra* L.), Siberian pine (*P. sibirica* Du Tour), and Siberian dwarf pine (*P. pumila* (Pallas) Regel) in the litter. Needles were sampled in pure or mixed pine forests in the NP Bohemian Switzerland (Czech Republic), along the rivers Timpton (Republica Sakha) and Vyerchni Sakujan (Zabaykalskaya Oblast) in Russia, and in the Windachtal Valley, Tyrol (Austria). Surface sterilisation of needles and cultivation conditions were identical to those described in Koukol (2010). Culture characteristics were observed on 2% malt agar (2MA, final sucrose content 2% w/v, 18 g agar, 1 l distilled water) prepared from brewer's wort (Staropramen Brewery, Prague, Czech Republic), potato carrot agar (PCA), oatmeal agar (OA), and potato-dextrose agar (PDA), all prepared from fresh ingredients according to Fassatiová (1986). Agar plates were maintained at laboratory temperature (22–25°C). For microscopy and measurements, conidiophores with conidia were mounted in Melzer's reagent and examined with phase or differential interference contrast (using an Olympus BX51 microscope with digital camera, measured using Quick Photo software). Pictures were further edited in Adobe Photoshop®. Microscopic measurements are reported as the mean $\pm$ standard deviation of 30–50 measurements with the extremes given in parentheses.

The holotype specimen is deposited in the PRM (National Museum, Prague, Czech Republic) and living cultures are maintained in the CCF (Culture Collection of Fungi, Prague, Czech Republic).

DNA extraction, amplification and sequencing

DNA was isolated from cultures grown on 2MA using the ZR Fungal/Bacterial DNA kit (Zymo Research, Orange, CA, USA). DNA analyses were made with sequences from the regions ITS1+5.8S+ITS2 (referred to it as the ITS rDNA) and 28S rDNA together with the gene coding for the translation elongation factor 1 α (EF-1 α). Amplification and sequencing were performed according to Koukol (2011) with the exception that only the shorter part of EF-1 α was amplified with primer pairs 983F a 1567R (Rehner & Buckley 2005). New sequences generated in this study from newly isolated strains are listed in TABLE 1. Sequences of ITS and 28S rDNA of *X. pinea* were obtained from

TABLE 1. Accession numbers of new sequences generated in this study.

SPECIES / CULTURE	ITS	28S rDNA	EF-1 α
<i>Infundichalara minuta</i>			
CCF4156, ex-type	HE603986	HE603981	HE603978
CCF4157	HE603987	HE603982	HE603977
CCF4158	HE603988	HE603983	HE603976
CCF4159	HE603989	HE603984	—
CCF4160	HE603990	HE603985	—
<i>Xenopolyscytalum pinea</i>			
CPC14225, ex-type	—	—	HE603980
CPC14234	—	—	HE603979

GenBank. The remaining sequences of *Chalara* spp., *Cistella acuum* (Alb. & Schwein.) Svrček, and *Infundichalara microchona* (W. Gams) Réblová & W. Gams originated in Koukol (2011).

Phylogenetic analyses

All markers were aligned using MUSCLE (Edgar 2004) and adjusted manually in BioEdit, v. 4.7.1 (Hall 1999) to maximize similarities. The dataset consisted of 32 sequences and comprised 1322 characters with 272 parsimony-informative sites. Phylogenetic analyses were conducted using MEGA v. 5 (Tamura et al. 2011), PhyML v. 3.0 (Guindon et al. 2010) and MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). For the Bayesian analyses, General Time Reversible model with invariant sites and a gamma rate distribution (GTR+I+G) was used due to its maximum complexity. The dataset was divided to three partitions (ITS, 28S rDNA and EF-1 α). The proportion of variable sites, rate frequencies and the gamma shape were estimated separately for the three partitions. Markov chains were initiated from a random tree and were run for 6,000,000 generations; samples were taken every 100th generation. Posterior probabilities (PP) were used as a Bayesian branch support on the consensus trees. In addition, bootstrap branch support (BS) values were estimated using the Maximum-Likelihood (ML) analyzes with 10,000 bootstrap replicates (MEGA 5.0, PhyML v. 3.0). The tree was rooted with *Tryblidiopsis pinastri* (Pers.) P. Karst. (AFTOL-ID 1319, sequences of 28S rDNA DQ470983, EF-1 α DQ471106).

Results

Five strains isolated during this study (CCF4156–4160) formed a strongly supported group sister to *I. microchona* (CBS175.74 and CBS889.73). The isolate B2S2 obtained from pine needles in Tyrol (Austria) clustered with *X. pinea* (Fig. 1). It most probably belonged to *X. pinea*, but was not morphologically observed, because it was lost due to contamination after DNA extraction. *Xenopolyscytalum pinea* (CPC14225 and CPC14234) clustered with low support with *Cistella acuum* (CCF3970).

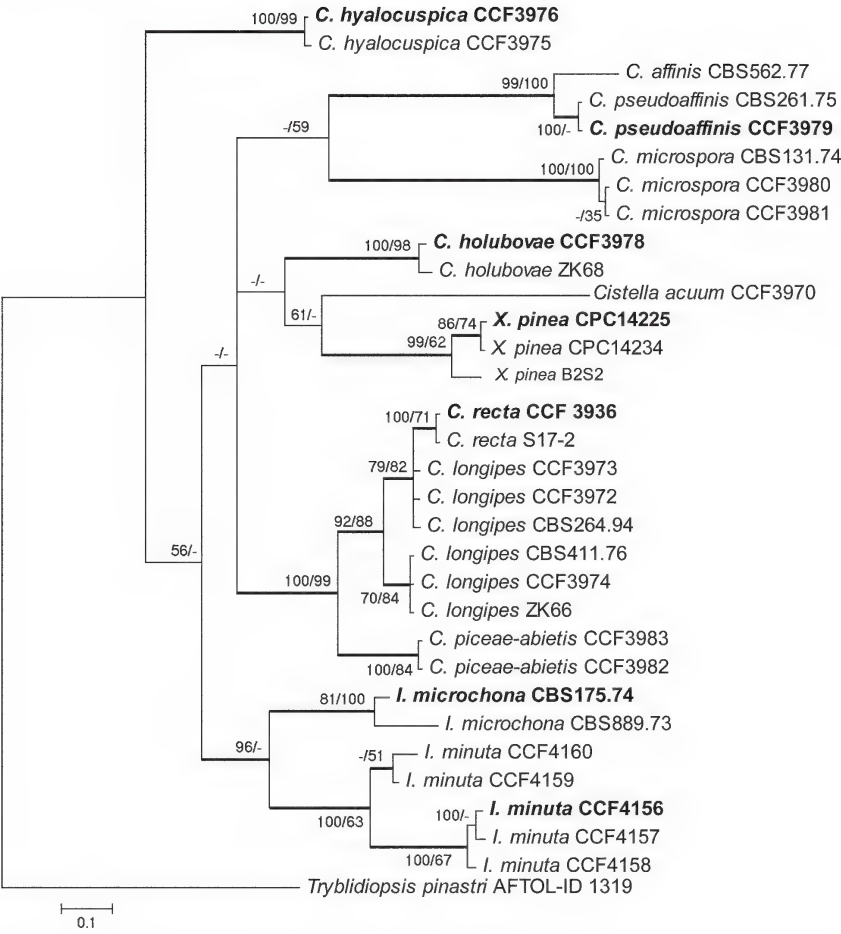


FIG. 1. Phylogenetic relationships of *Infundichalara minuta* and selected members of *Helotiales* derived from ITS, 28S rDNA and EF-1 α gene regions using ML and BA analyses. Genera are abbreviated as C. = *Chalara*, X. = *Xenopolyscytalum*, and I. = *Infundichalara*. Ex-type sequences are in bold. Bootstrap values > 50% are indicated along nodes. Thick lines show PP > 0.95. The tree was rooted with *Trybliopsis pinastri*.

Taxonomy

Infundichalara minuta Koukol, sp. nov.

FIGS 2, 3 a,b

MYCOBANK MB 563538

Differs from *Infundichalara microchona* by its smaller *Chalara*-like phialides producing wedge-shaped conidia with truncate base and synanamorph with penicillate conidiophores, ramoconidia, and subcylindrical conidia.

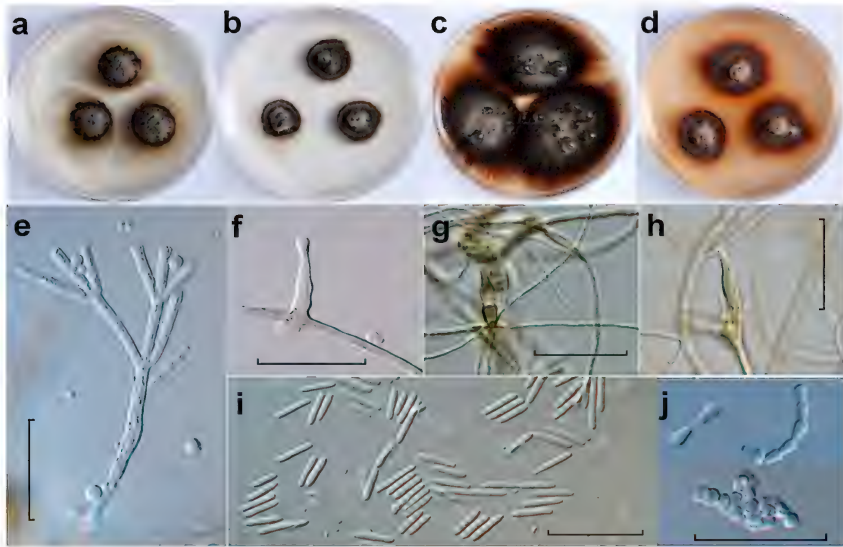


FIG. 2. *Infundichalara minuta*: colonial and microscopic morphology. a–d, Morphological variation on 2MA after 30 d incubation at 22–25°C; e, penicillate conidiophore with ramoconidia. f–h, *Chalara*-like conidiophores (f, intercalary; h, terminal); i, conidia produced on penicillate conidiophores; j, conidia produced from *Chalara*-like conidiophores—single, short chain, and cluster. (a,e,g,i,j = ex-type strain CCF4156; b = CCF4160; c,f,h = CCF4158; d = CCF4159. Bars = 20 µm.)

TYPE: Czech Republic, Bohemian Switzerland National Park, Doubice, Tokán, forest of *P. sylvestris* on sandstone rocks, 50°53.3'N 14°24.8'E, 450 m asl., on needles in the litter of *P. sylvestris*, 11 Dec 2006, leg. O. Koukol (Holotype, PRM899344; ex-type strain CCF4156).

ETYMOLOGY: *minutus* = referring to the minute *Chalara*-like conidiophores.

VEGETATIVE MYCELIUM consisting of smooth or warted, branched, septate, hyaline to pale brown hyphae 1.5–3 µm diam. CONIDIOPHORES dimorphic. PENICILLATE CONIDIOPHORES erect, producing branched, catenulate conidia visible as white tufts on needles and in culture, pale brown, 3–5-septate, ≤ 40 µm tall, 3–3.5 µm wide. CONIDIOGENOUS cells apical, hyaline to pale brown, smooth, 9.5–13.5(–15.5) × 1.5–3.5 µm, proliferating sympodially, usually giving rise to 3 ramoconidia. RAMOCONIDIA hyaline, smooth, aseptate, cylindrical to subcylindrical, (8–)9–13(–16) × 1.5–3 µm. CONIDIA subcylindrical, hyaline, smooth, aseptate, occurring in unbranched dispersible chains, ends with a flattened, slightly protruding scar, 0.8 µm wide, (5–)7–9.5(–11.5) × 1.5–2.5 µm. CHALARA-LIKE CONIDIOPHORES produced less frequently, formed either by conidiogenous cell on the mycelium or by conidiogenous cell on 1–4 basal cells,

that are erect, cylindrical, unbranched, pale brown, smooth, 4–10 µm tall (rarely ≤ 50 µm), 2.5–4 µm wide. CONIDIOGENOUS CELLS phialidic, hyaline to slightly pigmented, mostly formed terminally or intercalary directly on mycelium or on one supporting cell, subconical to lageniform, (8–)9.5–13.5(–20) × 3–4.5 µm, collarette mostly funnel-shaped, rarely cylindrical, 2.5–5 × 1.5–2.5 µm. CONIDIA wedge-shaped with truncate base, hyaline, smooth, 3–4(–5) × 2–3 µm, forming either short chains or clusters on top of the phialide.

COLONIES variable, different strains produced different morphologies in the dark, after 1 mo at 22–25°C (FIG. 2a–d). Colonies on 2MA eruptent in the centre, with dense aerial mycelium, surface dark gray to brown with pale margin; reverse pale gray to dark brown, reaching 8–28 mm diam. On PDA aerial mycelium developed, colonies gray to pale brown, protuberant in the centre, reaching 10–26 mm diam. On OA dendritic, slimy, aerial mycelium only at margins, surface dark gray to brown, reaching 17–22 mm diam. On PCA gray to pale brown, immersed, with protuberant centre, reaching 8–33 mm diam. The strain CCF4157 produced pale white immersed mycelium on OA, 2MA and PCA and diffused violet pigment into agar on PDA and reached ≤ 40 mm diam. on each agar medium tested. Most intensive sporulation of all strains was observed on 2MA.

ECOLOGY & DISTRIBUTION — colonizes needles in the litter and humus of *P. sylvestris*, *P. cembra*, *P. sibirica*, and *P. pumila*, in Czech Republic, Austria, Russia (this study) and Sweden (concluded from Lindahl et al. 2010).

ADDITIONAL SPECIMENS EXAMINED: AUSTRIA, TYROL, Windachtal Valley, sparse *P. cembra* forest above river, 46°57.57'N 11°02.61'E, 1828 m asl., on *P. cembra* needles in litter, 25 Jun 2010, leg. O. Koukol (PRM899346, living culture CCF4160). RUSSIA, REPUBLICA SAKHA, sparse growth of *P. sibirica* within *Larix gmelinii* (Rupr.) Rupr. on the right bank of river Timpton near the branching with Ojumrak, 57°11.3'N 126°6.6'E, 550 m asl., on *P. sibirica* needles in litter, 4 Aug 2010, leg. D. Svoboda (living culture CCF4157); sparse growth of *P. sibirica* within *L. gmelinii* on the right bank of river Timpton near the branching with Bolshoy Ulimakh 58°37.6'N 127°1.1'E, 270 m asl., *P. sibirica* needles in litter, 10 Aug 2010, leg. D. Svoboda (PRM899345, living culture CCF4158); ZABAYKALSKAYA OBLAST, *P. pumila* forest on the right bank of river Vyerchni Sakujan, 56°48.5'N 118°6.9'E, 766 m asl., on *P. pumila* needles in litter, 12 Aug 2010, leg. D. Svoboda (living culture CCF4159).

Discussion

Infundichalara minuta shares morphological characteristics with both *Infundichalara* and *Xenopolyscytalum*. It produces hyaline to slightly pigmented phialides with funnel shaped collarettes and conidia that are truncate on the base and rounded at the apex (FIG. 2f–h,j), which is consistent with *Infundichalara* (Rěblová et al. 2011). On the other hand, *Chalara*-like phialides and a *Polyscytalum*-like synanamorph with penicillate conidiophores (FIG. 2e,i) forming white tufts on the substrate are typical for *Xenopolyscytalum*

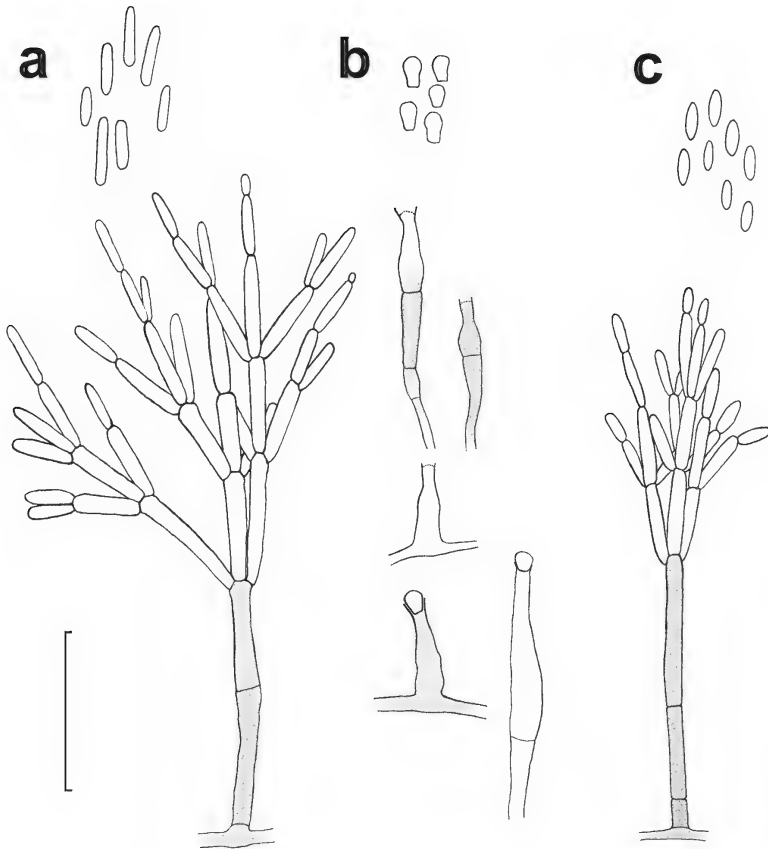


FIG. 3. *Infundichalara minuta* (ex-type strain CCF4156): a, Penicillate conidiophore and conidia; b, *Chalara*-like conidiophores and conidia. *Xenopolyscytalum pinea* (ex-type strain CPC14225): c, penicillate conidiophore and conidia. (Bar = 20 μ m).

(Crous & Groenewald 2010). Molecular data confirmed placement of the new species in *Infundichalara*, despite the closer morphological similarity with *Xenopolyscytalum*. Obviously, the presence of a *Polyscytalum*-like synanamorph is not phylogenetically significant at the genus level but may be used for recognizing the species (e.g., *C. holubovae* Koukol) that form typical *Chalara* phialides and produce a synanamorph with fusiform conidia (Koukol 2011).

The previously monotypic *Infundichalara* and *Xenopolyscytalum* represented by *I. microchona* and *X. pinea* respectively may be distinguished from *I. minuta*

based on morphology. *Infundichalara microchona* produces larger phialides ($18\text{--}35 \times 2.5\text{--}4.0\ \mu\text{m}$) but with shorter collarettes. The similarly sized phialoconidia of *I. minuta* and *I. microchona* differ in shape — wedge-shaped in *I. minuta* but clavate in *I. microchona* (Gams & Holubová-Jechová 1976). *Infundichalara minuta* phialides with the rather narrow collarette typical of *Chalara* s.str. are considered exceptional. Such variation has been noted as well for *I. microchona* (strain CBS125.74) with slightly longer collarettes (to $4\ \mu\text{m}$ deep; Gams & Holubová-Jechová 1976). Vice versa, molecular data support *C. longipes* (strain CBS867.73), which produces phialides with both types of collarettes, in *Chalara* (Koukol 2011). *Xenopolyscytalum pinea* is distinguished by shorter conidia formed on penicillate conidiophores ($3\text{--}4\text{--}(7) \times 1.5\text{--}(2)\ \mu\text{m}$ (FIG. 3c), larger *Chalara*-like phialides ($15\text{--}25 \times 2\text{--}3\ \mu\text{m}$), and cylindrical phialoconidia. Culture characteristics also differ, for *X. pinea* grew more quickly on all tested media (Crous & Groenewald 2010).

In this study, only freshly isolated cultures of *I. minuta* were surveyed. Pure cultures were obtained by picking conidia from penicillate conidiophores (white tufts on the surface-sterilised needles) in primary isolations after several weeks of cultivation. In pure cultures, this synanamorph also dominated and *Chalara*-like phialides were formed relatively rarely. Penicillate conidiophores and phialides were never observed growing from the same hyphae, but potential contamination of surveyed strains may be disproved as individual isolates were obtained independently from three distant localities.

It remains unknown whether *I. minuta* forms predominantly penicillate synanamorph also on field substrates. Similarly, Crous & Groenewald (2010) do not mention the prevalence of either form of *X. pinea* on natural substrates. The assumption that penicillate conidiophores may dominate on the substrate as well is indicated by the white colonies formed by an unknown *Polyscytalum*-like fungus that have been repeatedly found on Scots pine needles sampled close to the type locality of *I. minuta* and cultivated in damp chambers (Koukol 2007). Herbarium specimen “*Polyscytalum* sp. X07” (private herbarium of the author) sampled in September 2000 and surveyed in this study contained a fungus producing relatively dense growth of *Polyscytalum*-like conidiophores ($12\text{--}18 \times 2\ \mu\text{m}$) with chains of cylindrical, 0–1-septate, hyaline conidia ($5\text{--}13 \times 1.5\ \mu\text{m}$). Except for the septate conidia, its description matches *I. minuta*, but a fresh collection must first be isolated into pure culture and molecularly analyzed before it can be regarded as conspecific with *I. minuta*.

All *I. minuta* strains were obtained from pine litter, but the species also colonizes Scots pine humus according to 99% similarity (505/507 bp) of the ITS rDNA region with sequence GU559086 obtained in the study of Lindahl et al. (2010). *Infundichalara minuta* seems to have a negligible effect on degradation of structural polysaccharides in the litter. In a parallel study, strain CCF4156,

analyzed for the production of ten hydrolytic enzymes during submersed cultivation in a low-nitrogen medium, expressed only very low amounts of cellobiohydrolase and β -glucosidase (Koukol & Baldrian 2012).

Acknowledgments

I am very grateful to Dr. David Svoboda, who sampled litter needles on his journey across Russia, to Dr. Pedro Crous for sending me two strains of *X. pinea*. Dr. Pedro Crous and Dr. Roland Kirschner significantly contributed to the improvement of the manuscript as pre-submission reviewers. This study was supported by the Grant Agency of the Czech Republic, project No. 206/09/P295 and by the Ministry of Education, Youth and Sports of the Czech Republic (MSM0021620828).

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MYCOTAXON

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***Clavulicium hallenbergii*, a new corticioid species from India**

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ABSTRACT – A new corticioid species, *Clavulicium hallenbergii*, is described from Chamba, in Himachal Pradesh.

KEY WORDS – Manimahesh, *Pinus wallichiana*

During the fungal forays conducted in Dibri–Dhansho area in district Chamba of Himachal Pradesh, India, Avneet P. Singh collected a corticioid fungus on a trunk of *Pinus wallichiana*. Oil-rich clavate basidia and oil-rich basidiospores pointed in the direction of the genus *Clavulicium* (*Agaricomycetes*). After detailed comparison of macroscopic and microscopic characters with the relevant literature (Burt 1926, Parmasto 1968, Eriksson & Ryvarden 1973) we concluded that the fungus represents a new species in the genus *Clavulicium*, which herewith is described.

***Clavulicium hallenbergii* Avneet P. Singh, J. Kaur & Dhingra sp. nov.**

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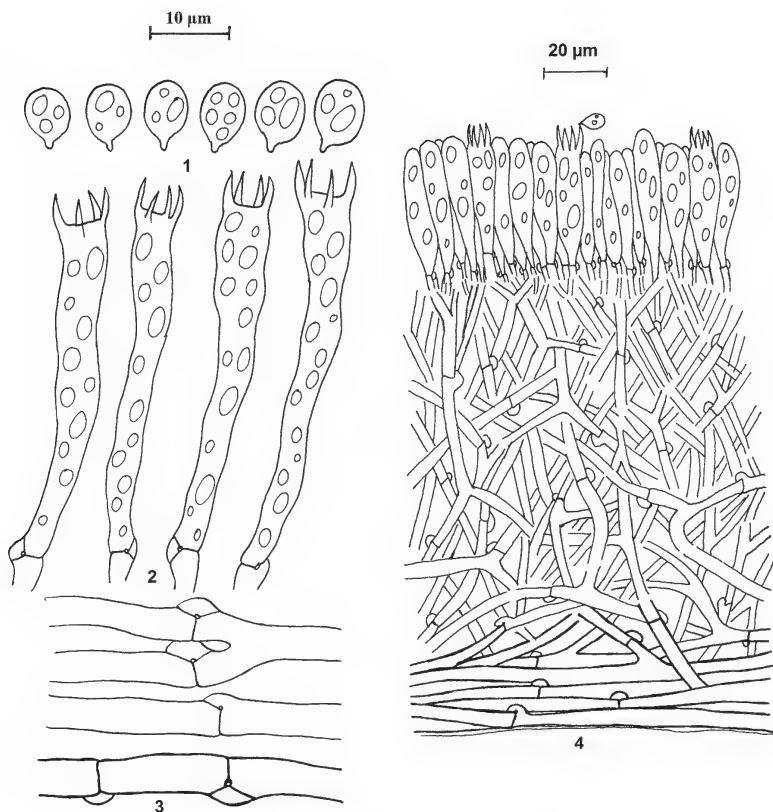
FIGS 1 – 5

Differs from *Clavulicium macounii* by its narrower basidia, smaller basidiospores, and lack of gloeocystidia.

TYPE: India, Himachal Pradesh: Chamba, Manimahesh, on way from Dibri towards Dhansho, on the trunk of *Pinus wallichiana* A.B. Jacks., 20 September 2005, Avneet 3683 (PUN, holotype)

ETYMOLOGY: In honour of the mycologist Prof. Nils Hallenberg, University of Gothenburg, Gothenburg, Sweden.

Basidiocarp resupinate, adnate, effused, up to 175 µm thick in section; hymenial surface smooth, brownish red to light brown; margins whitish, thinning, fibrillose, to indeterminate, loosening upon drying in mature



FIGS 1–4. *Clavulicium hallenbergii*, microscopic structures.

1. basidiospores; 2. basidia; 3. generative hyphae; 4. section through basidiocarp.

basidiocarp. Hyphal system monomitic; generative hyphae up to $3.0\ \mu\text{m}$ wide, branched, septate, clamped; basal hyphae somewhat thick-walled, parallel to the substrate; subhymenial hyphae thin-walled, vertically directed. Basidia $39.0\text{--}47.0 \times 3.0\text{--}5.0\ \mu\text{m}$, clavate, sinuous, 4-sterigmate, with oily contents and basal clamp; sterigmata up to $3.0\ \mu\text{m}$ long. Basidiospores $6.2\text{--}7.8 \times 4.5\text{--}5.5\ \mu\text{m}$, broadly ellipsoid to obovate, smooth, thin-walled, inamyloid, acyanophilous, with oil droplets.

REMARKS—*C. hallenbergii* resembles *C. macounii* (Burt) J. Erikss. & Boidin ex Parmasto in terms of morphology of the basidiocarp, clamped generative



FIG. 5. *Clavulicium hallenbergii*, basidiocarp (holotype).

hyphae and abundant oily contents in basidia and basidiospores. However, *C. macounii* differs in having gloeocystidia, wider basidia, and larger basidiospores ($9\text{--}12\text{ (}\pm 13\text{)} \times 6.5\text{--}8\text{ }\mu\text{m}$; Eriksson and Ryvarden, 1973).

Acknowledgements

Authors thank Prof. Nils Hallenberg (Evolutionary Botany, Botanical Institute, Gothenburg, Sweden) and Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review and Prof. Joost Stalpers (CBS Fungal Biodiversity Centre, Netherlands) for providing the Mycobank number. Head, Department of Botany, Punjabi University, Patiala, is thanked for providing research facilities.

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<http://dx.doi.org/10.5248/120.357>

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***Vararia longicystidiata* sp. nov. (Agaricomycetes) from India**

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ABSTRACT – A new corticioid species, *Vararia longicystidiata*, is described on decaying wood of *Quercus incana* from Uttarakhand state in India.

KEY WORDS – *Basidiomycota*, Chaurangi Khal, Uttarakashi

While conducting fungal forays in Chaurangi Khal area of district Uttarakashi, Uttarakhand (India), Samita collected an unknown corticioid fungus on decaying wood of *Quercus incana*. The presence of dichohyphidia and inamyloid smooth basidiospores indicated that the species belonged to *Vararia*, but it could not be assigned to any previously described species (Welden 1965, Parmasto 1970, Boidin & Lanquetin 1976, Rattan 1977, Boidin et al. 1980, Hallenberg & Eriksson 1985, Pegler & Young 1993). A detailed description of the specimen along with the microphotographs and line diagrams was sent to Prof. Nils Hallenberg (Sweden), who supported the description of a new species in the genus *Vararia*.

***Vararia longicystidiata* Samita, Sanyal, Dhingra & Avneet P. Singh, sp. nov.**

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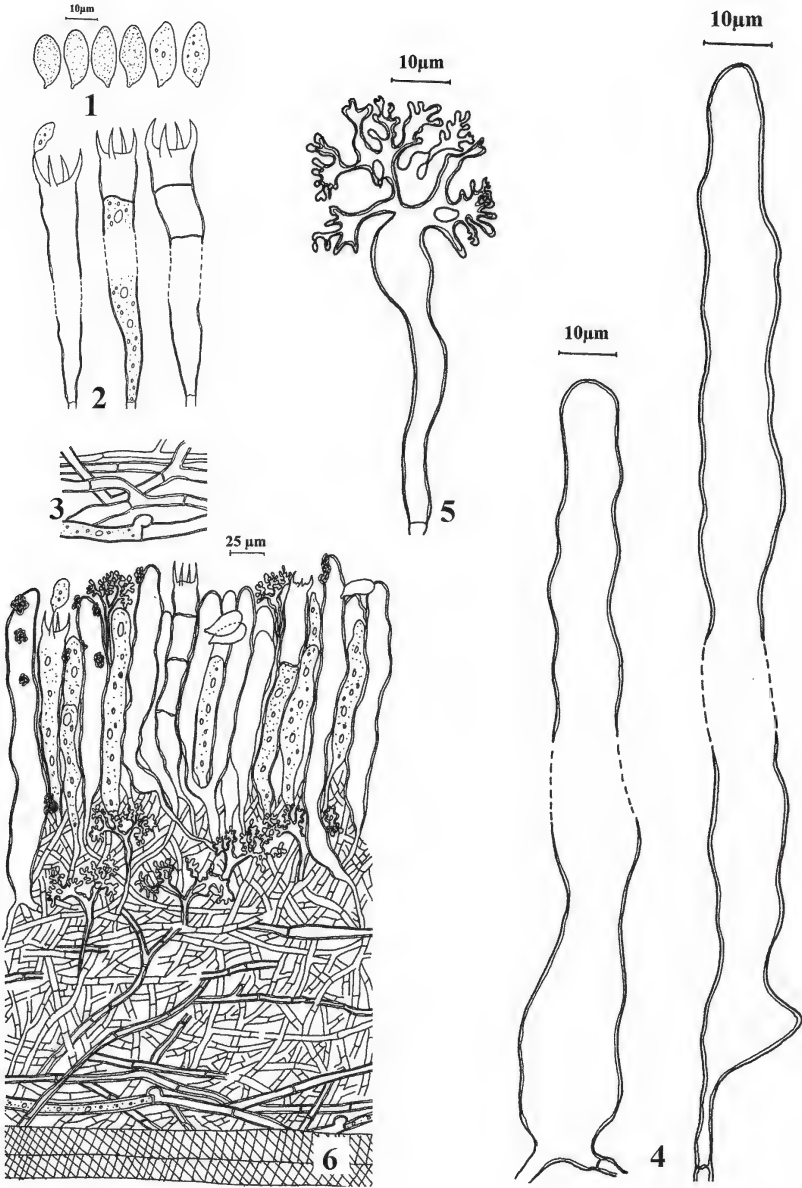
FIGS 1–7

Differs from *Vararia gomezii* in its larger cystidia, basidia, and basidiospores.

TYPE: India, Uttarakhand: Uttarakashi, Chaurangi Khal, on decaying wood of *Quercus incana* Bartram, 29 September 2011, Samita 4413 (PUN, **holotype**).

ETYMOLOGY: The epithet refers to the long cylindrical cystidia.

Basidiocarp resupinate, adnate, effused, up to 230 µm thick in section, hymenial surface smooth to somewhat tuberculate, light orange; margins thinning, fibrillose, paler concolorous to whitish. Hyphal system monomitic; generative hyphae up to 5.5 µm wide, branched, septate, generally without clamps; basal hyphae loosely arranged, more or less parallel to the substrate, interspersed with some thick-walled hyphae with oily contents, some cells



FIGS 1–6. *Vararia longicystidiata*, microscopic structures.
1. Basidiospores; 2. basidia; 3. generative hyphae; 4. cystidia;
5. dichohyphidia; 6. section through basidiocarp.

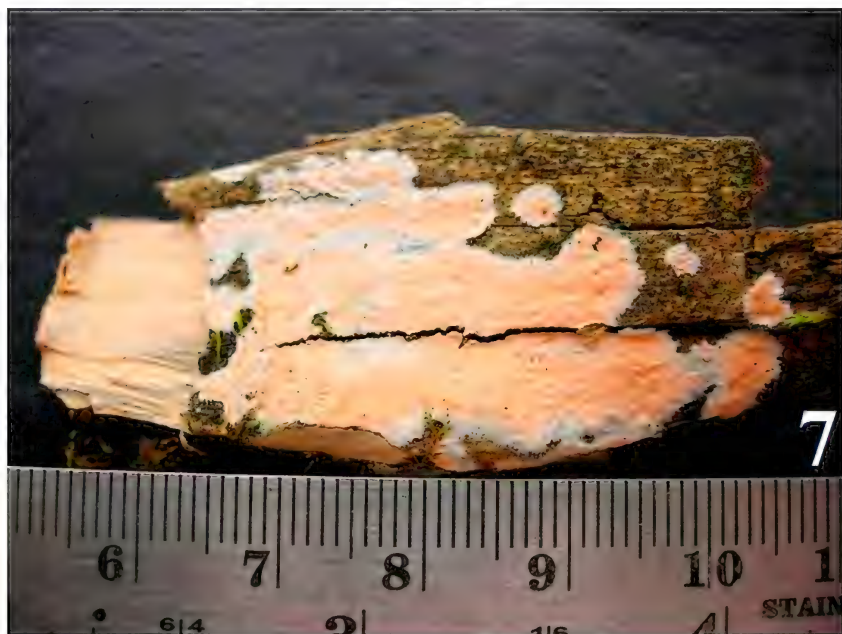


FIG. 7. *Vararia longicystidiata*, basidiocarp (holotype).

inflated; subhymenial hyphae vertical, compactly arranged. Dichohyphidia more common in subhymenium than hymenium, abundant, coralloid, branches irregular with blunt endings, dextrinoid. Cystidia $100.0\text{--}188.0 \times 8.8\text{--}17.5 \mu\text{m}$, abundant, subcylindrical to sinuous, thick-walled, without basal clamp, negative to sulphovanillin. Basidia $70.0\text{--}110.0 \times 9.0\text{--}13.0 \mu\text{m}$, clavate, 4-sterigmate, frequently with secondary septa, without basal clamp, with or without oily contents; sterigmata up to $12.5 \mu\text{m}$ long; basidioles rich in oily contents. Basidiospores $15.0\text{--}20.0 \times 6.0\text{--}7.5 \mu\text{m}$, sub-fusiform to navicular, with numerous oil-drops, thin- to somewhat thick-walled, inamyloid, acyanophilous.

REMARKS—*Vararia longicystidiata* resembles *V. gomezii* Boidin. & Lanq. (reported from South America and Africa) in having similar basidiocarps and basidiospore shape. However, *V. gomezii* differs in having ovoid unthickened cystidia ($14\text{--}50 \times 8\text{--}14 \mu\text{m}$), smaller basidia ($34.0\text{--}45.0 \times 7.5$), and smaller basidiospores ($13.0\text{--}14.8 \times 4.8\text{--}6.25 \mu\text{m}$; Boidin and Lanquetin; 1977).

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Gothenburg, Sweden) for expert comments and peer review; Prof. B.M. Sharma (Department of Plant Pathology, COA, CSKHPAU, Palampur, H.P., India) for peer review.

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A new cystidiolate variety of *Omphalina pyxidata* (Basidiomycota, tricholomatoid clade) from ItalyALFREDO VIZZINI^{1*}, MARIANO CURTI², MARCO CONTU³ & ENRICO ERCOLE¹¹*Dipartimento di Scienze della Vita e Biologia dei Sistemi - Università degli Studi di Torino
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ABSTRACT — A new variety of *Omphalina pyxidata*, var. *cystidiolata*, is here described and illustrated based on morphological and molecular data. The new combination *Infundibulicybe lateritia* is introduced.

KEY WORDS — *Agaricomycetes*, *Agaricales*, omphalinoid fungi, *Contumyces*, taxonomy

Introduction

Within the omphalinoid fungi — small agarics with a convex to deeply umbilicate pileus, central stipe, thin context, decurrent lamellae, white spore-print, and thin-walled inamyloid smooth spores (Norvell et al. 1994, Lutzoni 1997, Redhead et al. 2002a,b) — taxa with cystidiolate basidiomata are thus far known only in the hymenochaetoid clade (*Blasiphalia* Redhead, *Contumyces* Redhead et al., *Rickenella* Raithelh.; Moncalvo et al. 2000, 2002, Redhead et al. 2002b, Larsson et al. 2006, Larsson 2007).

During fieldwork focused on bryophilous *Galerina* species, we collected an omphalinoid fungus growing on mosses close to *Galerina discreta* E. Horak et al. We initially believed that these specimens represented a new species of *Contumyces* (Contu 1997, Redhead et al. 2002b) based on the presence of well-differentiated pileo-, caulo-, cheilo-, and pleurocystidia and an irregular hymenophoral trama. However an ITS-rDNA analysis implied they might represent an unknown as-yet undescribed variety of *Omphalina pyxidata* (Bull.) Quél., the type species of *Omphalina* Quél. (*Omphalina* s.s. = tricholomatoid clade sensu Matheny et al. 2006, Binder et al. 2010 and Vizzini et al. 2011a). This genus is characterized by bryophilous basidiomata with a reddish brown tinged

pileus and stipe, paler and well-developed lamellae, a smooth (not scaly) pileus, absence of hymenial and pileal cystidia, and presence of clamp-connections (Redhead et al. 2002a, Elborne 2008). We fully describe and illustrate the new taxon below.

Materials & methods

Morphology

The macromorphological descriptions follow the detailed field notes taken on fresh material. The micromorphological descriptions are based both upon study of fresh and herbarium material. Dried material was revived in 5% KOH and stained in Congo Red, Cotton Blue, and Melzer's reagent. The basidiospore range and means are based on measurements of 25 spores. In the macro- and microscopic descriptions Q = the quotient of spore length and width and Q_m = the average quotient; L = number of entire lamellae; l = number of lamellulae between each pair of entire lamellae. The basidial width was measured at the broadest part, and the length was measured from the apex (sterigmata excluded) to the basal septum. Colour codes in brackets (e.g., Se 45) follow Séguy (1936), hereafter referred to as (Se). Author citations follow the IPNI Authors and the Index Fungorum Authors of Fungal Names websites. Herbarium abbreviations follow Thiers (2011) with the exception of "EM" and "GT", referring to the personal herbaria of Enzo Musumeci and Gérard Trichies, respectively. The type collection is housed at TO (Herbarium of the Department of Plant Biology, University of Turin, Italy), and both name and Latin diagnosis of the new variety as well as the new combination are deposited in MycoBank (<http://www.mycobank.org>).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was isolated from 1 mg of 3 herbarium specimens (TABLE 1) using the DNeasy Plant Mini Kit (Qiagen, Milan Italy). Universal primers ITS1f/ITS4 were used for the ITS region amplification (White et al. 1990; Gardes & Bruns 1993). Amplification reactions were performed in PE9700 thermal cycler (Perkin-Elmer, Applied Biosystems) in a 25 µl reaction mixture using the following final concentrations or total amounts: 5 ng DNA, 1× PCR buffer (20 mM Tris/HCl pH 8.4, 50 mM KCl), 1 µM of each primer, 2.5 mM MgCl₂, 0.25 mM of each dNTP, 0.5 unit of *Taq* polymerase (Promega). The PCR program was as follows: 3 min at 95°C for 1 cycle; 30 s at 94°C, 45 s at 50°C, 2 min at 72°C for 35 cycles, 10 min at 72°C for 1 cycle. PCR products were resolved on a 1.0% agarose gel and visualized by staining with ethidium bromide. PCR products were purified and sequenced by DiNAMYCODE srl (Turin, Italy). Sequence assembly and editing were performed using Geneious v5.3 (Drummond et al. 2010). The sequences are deposited in GenBank under the accession numbers given in TABLE 1.

Sequence alignment and phylogenetic analysis

Sequences included in the phylogenetic analyses were either generated in this study (TABLE 1) or retrieved from GenBank. Multiple sequence alignments for ITS fragments were generated using MAFFT (Katoh et al., 2002) with default conditions for gap opening and gap extension penalty. The alignment was slightly edited using MEGA 5.0 (Tamura et al. 2011). Phylogenetic analyses were performed using both Bayesian Inference (BI)

TABLE 1. *Omphalina pyxidata* collections used in this study.

COLLECTIONS	COLL. NO., COUNTRY, DATE, COLLECTOR	ITS Acc. No.
* <i>O. pyxidata</i> 1	GT99398, France, 02/12/1999, G. Trichies	JQ671000
* <i>O. pyxidata</i> 2	EM0434-05, Switzerland, 06/07/2005, E. Musumeci	JQ671001
<i>O. pyxidata</i> 3	TO AV98, Italy, 10/10/2010, A. Vizzini	JN944402
<i>O. pyxidata</i> 4	Lamoure L66-118hl4, culture (see Lutzoni 1997)	OPU66450
* <i>O. pyxidata</i> var. <i>cystidiata</i>	TO HG2512, Italy, 11/11/2010, M. Curti	JQ671002

* = collections newly sequenced in this study.

and Maximum Likelihood (ML) approaches. The BI was performed with MrBayes 3.1.2 (Huelsenbeck & Ronquist 2001) with four incrementally heated simultaneous Monte Carlo Markov Chains (MCMC) ran over 10 millions generations, under GTR+I evolutionary model. Trees were sampled every 1000 generations resulting in an overall sampling of 10,001 trees; the first 2500 trees were discarded as “burn-in” (25%). For the remaining trees, a majority rule consensus tree showing all compatible partitions was computed to obtain estimates for Bayesian Posterior Probabilities (BPP). Branch lengths were estimated as mean values over the sampled trees. ML was performed with RAXML (Stamatakis 2006) under GTRGAMMA model and using thorough bootstrap with 20 runs and 1000 replicates. In both analyses a *Pseudoclitocybe cyathiformis* sequence (Genbank HM191730) was used as outgroup taxon, according to Binder et al. (2010) and Vizzini et al. (2011a). The ML consensus tree was used merely for comparison with the Bayesian tree and to support the analysis. However the BPP (Bayesian posterior probability) over 0.75 and the ML bootstrap (MLB) over 50% values are reported in the resulting tree. Pairwise % identity values of ITS sequences were calculated using MEGA 5.0 (Tamura et al. 2011).

Results

Molecular results

Bayesian and Maximum likelihood inferences were performed on a total of 18 samples, including 15 sequences available from GenBank and three newly sequenced specimens (TABLE 1). Final alignment length was 731 bp. Both Bayesian and Maximum likelihood analyses produced the same topology (FIG. 1). In both analyses our cystidiate collection clusters with four *O. pyxidata* sequences clearly forming a strongly supported clade (BPP 0.92 and MLB 85%). The five *O. pyxidata* sequences show a pairwise % identity value of 99.8 and could be considered conspecific.

The *O. pyxidata* sequence deposited in Genbank under the accession number JF908502 (MCVE 15669) was not used in the analysis because a Blastn search

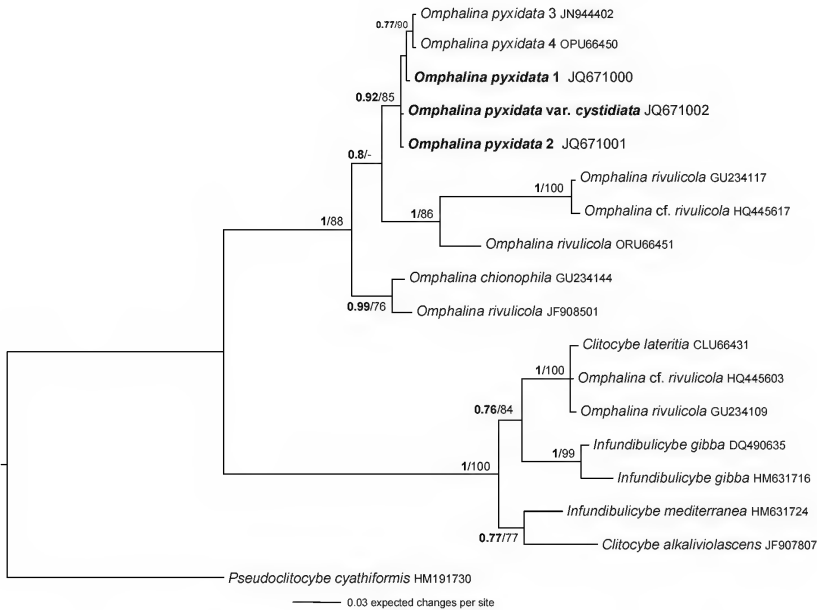


FIGURE 1. Bayesian phylogram obtained from the ITS sequence alignment. Support values for clades that are supported in either the Bayesian (Posterior Probabilities values – BPP) and Maximum likelihood (ML Bootstrap percentage – MLB) analyses are indicated. BPP above 0.75 and MLB above 50% are given above branches. Taxa in bold represent newly sequenced collections. Numbers (1–4) refer to the collections reported in TABLE I.

implies it represents a non-omphalinoid species in the *Pluteus cinereofuscus*/*P. nanus* complex.

Clitocybe lateritia, together with two *O. rivulicola* (J. Favre) Lamoure collections, clearly falls within *Infundibulicybe*. *Clitocybe alkaliviolascens* Bellù clusters close to *Infundibulicybe mediterranea* Vizzini et al.

Taxonomy

Omphalina pyxidata var. *cystidiata* M. Curti, Contu & Vizzini, var. nov. FIGS 2–3

MYCOBANK MB564484

A varietate typica differt praesentia cystidiorum in lamella, pileo, et stipite. Habitat: gregatim, ad muscos.

TYPE — Italy, Latium, prov. Rieti, com. Pozzaglia Sabina, Valle del Turano, 42.1596°N 12.9652°E, 878 m, 11.XI.2010, leg. M. Curti (TO HG2512 **holotype**).

ETYMOLOGY — the specific epithet refers to the presence of hymenial as well as of pileo- and caulocystidia.



FIGURE 2. *Omphalina pyxidata* var. *cystidiata*. Basidiomata. Bar = 20 mm.

PILEUS 3–15 mm broad, at first convex to applanate, later with a slightly depressed centre, not or slightly hygrophanous, minutely pubescent, dark brown-fulvous (Se 96, 121, 126, 146, 251, 252) fading to light brown (Se 133, 134, 173); margin at first involute then expanding, smooth to slightly striate in old basidiomes, whitish in dried basidiomes. LAMELLAE ($L = 18\text{--}25$; $l = 1\text{--}2$) decurrent, thick, distant with lamellulae occasionally interspersed, strongly forked and intervenose, whitish to light ochre (Se 199–200), with entire concolorous edge. STIPE 15–40 $\times$ 1–3 mm, cylindrical, slightly broadened at the base, sinuous-flexuous, solid then fistulous, cartilaginous, paler than pileus, light ochre-brown (Se 133, 174, 203–204), minutely pruinose to subglabrous. CONTEXT elastic, thin, whitish-ochre to light ochre-brown (Se 133, 134) in surface; smell and taste not distinctive. SPORE PRINT white.

SPORES (7–)7.5–9(–10) $\times$ 4.5–6(–6.5) μm , $Q = 1.3\text{--}1.7$, $Q_m = 1.51$, largely ellipsoid to oblong in frontal view, lacrymoid to slightly amygdaliform in side-view, smooth, thin-walled, hyaline, inamyloid, mono-biguttulate, with a thick and evident hilum. BASIDIA 28–46(–60) $\times$ 6–9 μm , clavate, (two-) four-spored, sterigmata 3–7 μm long, usually clamped. CHEILOCYSTIDIA 23–45 $\times$ 3–6 $\times$ 2.5–5 μm , abundant, cylindrical, slender, apically tapered or slightly swollen, some laterally ventricose, hyaline, thin-walled, often with inner microguttules. PLEUROCYSTIDIA 25–50(–65) $\times$ 3.5–5.5 $\times$ 2.5–5(–7) μm , similar to cheilocystidia, but slightly longer. HYMENOPHORAL TRAMA irregular, consisting of 3.3–7 μm wide hyphae. PILEIPELLIS a cutis consisting of cylindrical, interwoven, 6–10

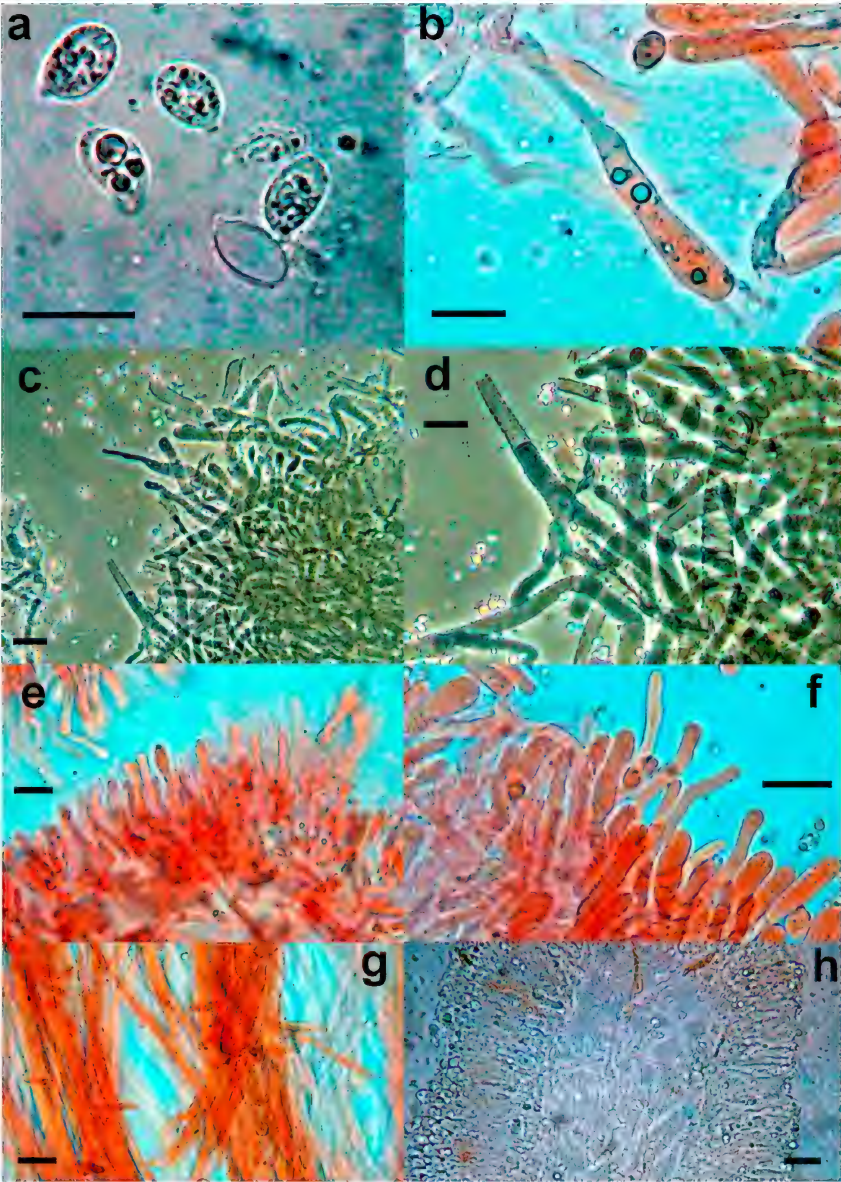


FIGURE 3. *Omphalina pyxidata* var. *cystidiata*. Microscopic features. a. Spores showing protruding hilum. b. Basidium with basal clamp. c. Pileipellis and pileocystidia. d. Encrusting pigment of pileipellis elements. e. Cheilocystidia (with inner microguttules). f. Pleurocystidia. g. Caulocystidia. h. Irregular hymenophoral trama. Bars: a–d = 10 μ m; e–h = 20 μ m.

µm wide hyphae; terminal elements with a subclavate 7–13 µm wide apex; subcutis made up of 3–7 µm wide hyphae; PIGMENT epiparietal, minutely to strongly encrusting; PILEOCYSTIDIA numerous, fusiform to lageniform, 35–60 × 3.5–9 × 3.5–5 µm. STIPITPELLIS a cutis made up, in 5% KOH, of ochre-yellow 3–8 µm wide hyphae bearing minute incrustations; CAULOCYSTIDIA similar to hymenial cystidia (30–65 × 4–5 × 3–5 µm). STIPITITRAMA non-sarcodimitic, consisting of hyphae up to 16 µm wide. CLAMP CONNECTIONS present at all septa. THROMBOPLEROUS HYPHAE (= oleiferous hyphae sensu Cléménçon 2004) present. APPRESSORIA on host surface not observed.

HABITAT — Gregarious, rarely subcaespitose, on *Tortula muralis* gametophytes (*Bryophyta*).

ADDITIONAL MATERIAL STUDIED — *Omphalina pyxidata* var. *pyxidata*: FRANCE, LORRAINE, MOSELLE, Moyeuve-Petite, 02 Dec 1999, on mosses, leg. G. Trichies (GT99398); SWITZERLAND, CANTON BASEL STADT, Lange Erlen, 06 Jul 2005, on mosses, 250 m asl, leg. E. Musumeci (EM0434-05); ITALY, PIEDMONT, CHISONE VALLEY, Pinerolo, Prà Martino, 10 Oct 2010, on mosses, 1000 m asl, leg. A. Vizzini (TO AV98).

Discussion

As highlighted by Vizzini et al. (2011a), *Omphalina* s.s. is sister to *Infundibulicybe* Harmaja, a genus recently segregated from *Clitocybe* (Fr.) Staude (Harmaja 2003). In the six-gene region sequence analyses by Matheny & al. (2006), *Infundibulicybe* falls outside the tricholomatoid clade, occupying an isolated position in the *Agaricales* (incertae sedis). However, recent analyses by Binder et al. (2010), Vizzini et al. (2011a) and Matheny (pers. comm.) place it with robust support as sister to the rest of the tricholomatoid clade. *Omphalina* and *Infundibulicybe* share the cream-reddish brown tinges of pileus and stipe, the long-decurrent lamellae, and strongly encrusting pigment (Harmaja 2003, Vizzini et al. 2011b).

Our molecular analysis (FIG. 1) and morphological comparison support the new taxon as a cystidiolate variant of *O. pyxidata* thus far never described. *Omphalina pyxidata* var. *cystidiata* is unique within *Omphalina* s.s., where it is the only taxon thus far included that possesses well-developed cheilo-, pleuro-, pileo-, and caulocystidia (Bigelow 1970, 1985, Singer 1970, Lamoure 1974, 1975 1982; Cléménçon 1982, Bon 1997, Elborne 2008). Furthermore, we have found no cystidia in three examined European collections of *O. pyxidata* (see ADDITIONAL MATERIAL STUDIED). Morphologically, our new taxon resembles the omphalinoid bryophilous genera belonging to the hymenochaetoid clade (Redhead et al. 2002b, Antonín & Noordeloos 2004). However, *Rickenella* differs in having a regular hymenophoral trama (Contu 1997, Redhead et al. 2002b) and *Blasiphalia* by growing specifically on the *Blasia pusilla* gametophyte (*Marchantiophyta*) and forming appressoria on host surface (Larsson et al.

2006). Apart from molecular data that clearly support our new variety within the *Agaricales*, morphologically *Contumyces* (= *Jacobia* Contu, nom. illeg.; *Hymenochaetales*) might accommodate our taxon. *Contumyces* so far comprises only three species — *C. rosellus* (M.M. Moser) Redhead et al., *C. vesuvianus* (F. Brig.) Redhead et al., and *C. brunneolilacinus* (Contu et al.) Redhead et al. (Redhead et al. 2002b, Antonín & Noordeloos 2004) — but only the first named has been sequenced. *Contumyces rosellus* differs from *Omphalina pyxidata* var. *cystidiata* by the pink to pale salmon-pink colouration, white stipe, and longer and larger cystidia. *Contumyces vesuvianus* has orange to fulvous basidiomata, larger basidiospores, rarer differently shaped (mucronate/rostrate) cystidia and intracellular pigment in the pileipellis hyphae. As originally described (Contu 1989), *C. brunneolilacinus* differs in having a lilaceous-brown, sharply tomentose, hygrophanous pileus, pink-lilac lamellae, a *Pelargonium*-like smell, lageno-capitate cystidia, and intracellular pigment in the pileipellis hyphae. Finally, *C. brunneolilacinus* sensu Antonín & Noordeloos (2004) differs in having longer and larger cystidia and a non-tomentose pileal surface.

Our sequence analyses have also revealed that *Clitocybe lateritia* clusters in *Infundibulicybe* with two collections clearly misdetermined as *O. rivulicola* (Fig. 1). The three sequences are clearly conspecific (pairwise % identity value = 99.7). The two “*O. rivulicola*” sequences were derived from soil or ectomycorrhizal samples sampled from *Dryas octopetala* beds, as reported in two recent papers dealing with alpine-arctic fungi (Bjorbækmo et al. 2010, Geml et al. 2012). *Clitocybe lateritia*, a rare alpine-arctic species strictly associated with *Dryas octopetala* (*Rosaceae*), should be transferred to *Infundibulicybe* based on both sequence analyses and its *Infundibulicybe*-like features (reddish-brown colouration, strongly encrusting pigment in the pileipellis, partly lacrymoid spores) as inferred from Favre (1955), Gulden & Jennsen (1988), and Bon (1997). Consequently, we propose the following new combination:

***Infundibulicybe lateritia* (J. Favre) Vizzini & Contu, comb. nov.**

MYCOBANK MB564485

= *Clitocybe lateritia* J. Favre, Ergebn. wiss. Unters. schweiz.

Natn. Parks, n.s. 5(33): 54, 199 (1955).

TYPE: Switzerland, Munt la Schera, 2400 m a.s.l., in *Dryas* beds, 21.08.1941, leg. J. Favre

(Lectotype designated here, G-K16433; specimen illustrated in Favre 1955: Fig. 34; Pl. IV, Fig. 11).

Finally, *Clitocybe alkaliviolascens*, a species of the *Infundibulicybe gibba* complex recently described from Mediterranean areas and characterized by a dark brown pileus surface turning pink-violet with a 30% KOH drop, and spores reaching 10 µm in length (Bellù 1995, 1996), also belongs in *Infundibulicybe*. Bellù will propose the new combination in a forthcoming paper (Bellù, pers. comm.).

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Four new records of *Aspergillus* sect. *Usti* from Shandong Province, China

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ABSTRACT—Four new Chinese records of *Aspergillus* species: *Emericella heterothallica*, *Aspergillus calidoustus*, *A. keveii*, and *A. pseudodeflectus* are reported from Shandong Province. *Emericella heterothallica*, despite being uncommonly reported, should be regarded as geographically widely distributed.

KEY WORDS — morphology, sequence analysis, taxonomy

Introduction

Raper & Fennell (1965) recognized five species in the *Aspergillus ustus* group for which Gams et al. (1985) later established subgen. *Nidulantes* sect. *Usti*. Kozakiewicz (1989) added two species to this section and removed one species, while Klich (1993) transferred *A. granulosus* Raper & Thom from sect. *Usti* to sect. *Versicolores*. The phenotypic evidence spawned different opinions on phylogenetic relationships.

Peterson (2000) rejected sect. *Usti* based on LSU rDNA sequence data (with low bootstrap support) and placed the species in sect. *Nidulantes*. Houbraken et al. (2007), who revived *A. insuetus* (Bainier) Thom & Church based on multilocus DNA data along with chemical and phenotypic evidence, accepted eight species in sect. *Usti*; Peterson (2008), accepting the species Houbraken et al. included, added seven additional species to the section. Recently, Samson et al. (2011) added five new taxa to this section, enlarging to twenty-one but without including *A. ochraceoroseus* and *A. versicolor*.

Only three species representing sect. *Usti* have thus far been recorded in China: *A. deflectus* Fennell & Raper, *A. puniceus* Kwon-Chung & Fennell, and *A. ustus* (Qi et al. 1997). In the present paper, we report the recovery of four additional members of this section: *E. heterothallica*, *A. calidoustus*, *A. keveii*,

TABLE 1. Strains included in molecular phylogenetic analyses.

SPECIES	STRAINS *	GENBANK ACCESSION NUMBERS ^		
		ITS1-5.8S-ITS2	Ben A	CaM
<i>Aspergillus amylovorus</i> Panas. ex Samson	NRRL5813 T	EF652503	EF652327	EF652415
<i>A. calidoustus</i>	CBS 114380	EF591741	EF591729	EF591716
	CBS 113228	EF591739	EF591730	EF591715
	AS3.15302	JN982696	JN982686	JN982676
<i>A. deflectus</i>	NRRL2206 T	EF652437	EF652261	EF652349
<i>A. egyptiacus</i> Moub. & Mustafa	NRRL5920 T	EF652504	EF652328	EF652416
<i>A. elongatus</i> J.N. Rai & S.C. Agarwal	NRRL5176 T	EF652502	EF652326	EF652414
<i>A. granulosus</i>	NRRL 1932 T	EF652430	EF652254	EF652342
<i>A. insuetus</i>	CBS 107.25 T	EU076356	EU076371	EU076366
	CBS 119.27	EU076355	EU076372	EU076367
<i>A. keveii</i>	NRRL 1974	EF652432	EF652256	EF652344
	CBS 561.65	EU076352	EU076375	EU076364
	CBS 209.92 T	EU076354	EU076376	EU076365
	AS3.15305	JN982704	JN982694	JN982684
<i>A. lucknowensis</i> J.N. Rai et al.	NRRL 3491 T	EF652459	EF652283	EF652371
<i>A. minutus</i> Abbott	NRRL 4876 T	EF652481	EF652305	EF652393
	NRRL 279	EF652457	EF652281	EF652369
<i>A. pseudodeflectus</i>	NRRL6135 T	EF652507	EF652331	EF652419
	NRRL278	EF652456	EF652280	EF652368
	AS3.15306	JN982697	JN982687	JN982677
	AS3.15307	JN982700	JN982690	JN982680
	AS3.15308	JN982699	JN982689	JN982679
	AS3.15309	JN982701	JN982691	JN982681
<i>A. puniceus</i>	AS3.15310	JN982703	JN982693	JN982683
	NRRL 5077 T	EF652498	EF652322	EF652410
<i>A. ustus</i>	NRRL 4991	EF652492	EF652316	EF652404
	NRRL 275 T	EF652455	EF652279	EF652367
	AS3.15311	JN982695	JN982685	JN982675
	AS3.15312	JN982702	JN982692	JN982682
<i>A. versicolor</i> (Vuill.) Tirab.	NRRL238 T	EF652442	EF652266	EF652354
<i>Emericella heterothallica</i>	NRRL 5096 T	EF652449	EF652323	EF652411
	NRRL 5097	EF652500	EF652324	EF652412
	AS3.15313	JN982698	JN982688	JN982678

* Ex-type strains are indicated with “T”.
^ Sequences JN982675–JN982704 were obtained in the present study.

and *A. pseudodeflectus*. All were isolated from soil samples collected at the foot of Mount Tai in Shandong Province, located in the monsoon area of moderate-temperate zone of China (36°15'17"N 117°06'15"E) in late July 2011. The average altitude of that area is 134 m with an atmospheric pressure of 1004.1

hPa; the annual average temperature is 12.8 °C, with the monthly average of −1.4 °C in January and 26.5 °C in July; the soil is typically frozen from late October to late March; the frost-free period is about 200 days from late March to late September; the annual precipitation is 700 mm (<http://www.weather.com.cn>).

Materials & methods

Soil samples were collected underneath leaf litter and kept in sterilized plastic bags. The fungi were isolated through dilution plating (Malloch 1981) using Dichloran Rose Bengal Chlorotetracycline agar (King et al. 1979) as the selective medium. Ten sect. *Usti* strains were obtained and deposited in the China General Microbiological Culture Collection Center, Institute of Microbiology, Chinese Academy of Sciences, Beijing (CGMCC).

Morphological characters were assessed according to Houbraken et al. (2007), Klich (2002), and Raper & Fennell (1965). Color names (shown here in title case) follow Ridgway (1912). Wet mounts were prepared using material from colonies grown on Czapek yeast autolysate agar (CYA) at 25 °C after 14 days mounted in lactophenol without dye (Raper & Fennell 1965). Optical microscopic examination and photography were performed with a Nikon Eclipse 80i and Nikon Digital Sight DS-L1 Microscope (Nikon Co. Ltd., Japan).

Total DNA extraction followed the method of Scott et al. (2000). For amplification of a portion of the β -tubulin gene, we used the primers described by Glass & Donaldson (1995); to amplify the ITS1-5.8S-ITS2 region of the NUC rRNA gene, we used the primers designed by White et al. (1990). To amplify a portion of the calmodulin gene, we used the following primers: cmdAD1 5'-GCC GAC TCT TTG ACT GAA GAG C-3', cmdAD2: 5'-GCC GAT TCT TTG ACC GAG GAA C-3' and cmdAD3: 5'-GCC GAT TCT TTG ACC GAA GAA C-3' (sense primers); cmdQ1: 5'-GCA TCA TGA GCT GGA CGA ACT C-3' and cmdQ2: 5'-GCA TCA TGA GCT GGA CGA ATT C-3' (antisense primers). Polymerase chain reaction (PCR) protocols for amplification of the above three gene regions, and the purification and sequencing of PCR products followed the methods of Wang & Zhuang (2007).

Raw sequences were assembled and edited manually with BioEdit 5.0.9 (Hall 1999). Edited sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and adjusted manually, as needed. Thirty-three strains of sect. *Usti* (TABLE 1) were analyzed using the neighbor-joining (NJ) method and subjected to 1000 bootstrap replicates. All the phylograms yielded the same results (e.g., FIG. 5, based on partial calmodulin gene sequences).

Taxonomy

Emericella heterothallica (Kwon-Chung, Fennell & Raper) Malloch & Cain PL. 1

Colonies on Czapek agar (CA) at 25 °C growing rapidly, Colonies in 14 days reaching 40–43 mm in diam., densely floccose to velvety, radially sulcate, margins entire; Hülle cells aggregated into small white masses visible to the unaided eye, distributed sparsely on the colony surfaces; conidiogenesis sparse, only present in the adjacent areas of colonies, brown in color, similar to *Verona*

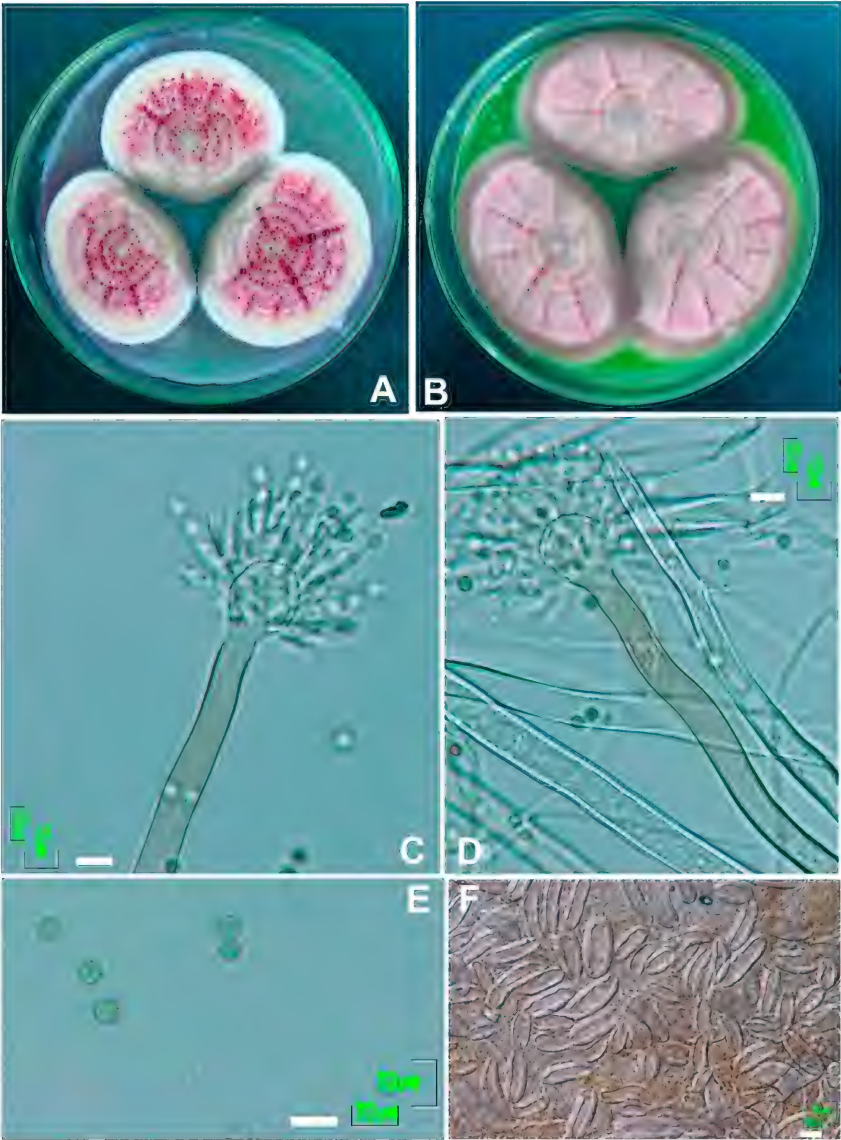


PLATE 1. *Emericella heterothallica* (AS3.15313): A–B. colonies on CA, CYA at 25 °C after 14 days; C–D. conidiophores; E. conidia; F. hülle cells. Bar = 10 µm

Brown, mycelia Orange-Cinnamon to Vinaceous-Cinnamon, white at margins; exudate abundant, Brazil Red to Strawberry Pink; soluble pigment abundant, Congo Pink; reverse Indian Red.

Colonies on CYA at 25 °C growing very rapidly, attaining 50–53 mm in diam. in 14 days; texture velvet, radially sulcate with lightly discernable annular plications, margins entire; Hülle cells moderately abundant, aggregated into conspicuous, small white masses, distributed sparsely on the surfaces; conidiogenesis moderate, in marginal areas, Verona Brown; mycelia Cinnamon to Vinaceous-Cinnamon, white at colony peripheries; exudate light yellow brown, moderately abundant; soluble pigment Empire Yellow, moderately abundant; reverse Mars Orange to Orange Rufous.

Conidial heads globose when young, splitting at maturity into 2–3 loosely divergent, short columns, (100–)120–180 µm long; conidiophores arising from substratum or surface hyphae, stipes colorless becoming light brown in upper portion, thin-walled, 240–360(–420) × (5–)7–9(–11) µm; vesicles subglobose to ellipsoidal, thin-walled, 14–18(–22) × 11–14 µm, biseriate; metulae 5–6.5 × 2–3 µm; phialides ampuliform, 6.5–7 µm, with short collula; conidia globose (3–)3.5–5.5 µm, echinulate, brown-colored en masse; Hülle cells ellipsoidal to very elongate, or joined into botuliform chains, thick-walled, commonly 36–45 × 9–16 µm, smaller ones 16–27 µm, longer ones up to 54–63 µm.

Colonies on CYA with 20% sucrose (CY20S) at 25 °C growing very rapidly, reaching 52–55 mm in diam. in 14 days, densely floccose to velvety, radially sulcate with annular plications, margins entire; conidiogenesis absent; mycelia Ochraceous-Buff, white at margins; exudate and soluble pigment absent; reverse Nopal Red.

Colonies on CYA at 37 °C growing moderately, in 14 days attaining 36–38 mm diam.; texture velvety, flat, margins entire; conidiogenesis moderately heavy in central areas, brown-colored near Bister, with a Mikado tinge; mycelia white at margins to Baryta Yellow in submarginal areas; exudate and soluble pigment absent; reverse Lemon Chrome, with Buff Yellow tinge in centers.

ISOLATE EXAMINED: China, Shandong Province, foot of Mount Tai, from soil, 23 July 2011, Z. Yang (CGMCC AS3.15313).

Aspergillus calidoustus Varga, Houbraken & Samson

PL. 2

Colonies on CA at 25 °C growing moderately, colonies attaining 35–40 mm in diam. in 14 days, flat, sparsely floccose, margins fimbriate; conidiogenesis sparse, Light Drab; mycelia white; exudate and soluble pigment absent; reverse white.

Colonies on CYA at 25 °C growing rapidly, attaining 48–49 mm in diam. in 14 days, moderately deep, margins entire, floccose; conidiogenesis heavy, Drab Gray to Light Drab; mycelia white; exudate and soluble pigment absent; reverse Pale Yellow–Green to Light Viridine Green.

Conidial heads small, radiate; conidiophores borne from aerial hyphae, stipes straight, with light yellow tinge, smooth-walled, (130–)150–250(–300)

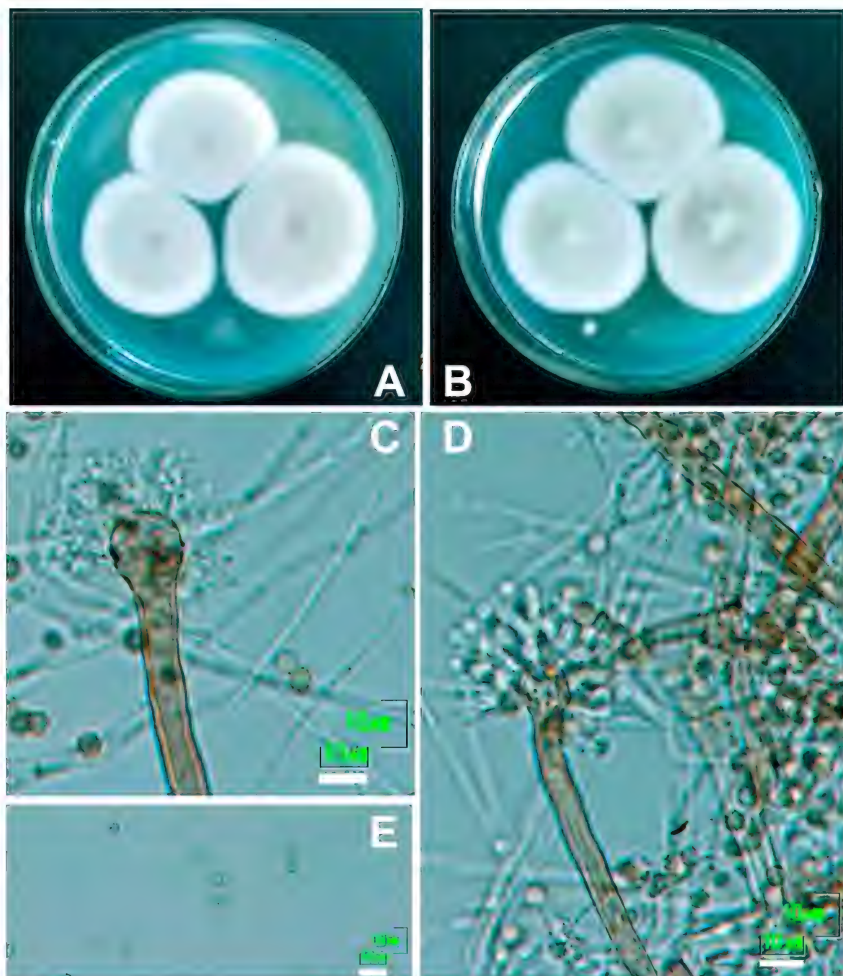


PLATE 2. *Aspergillus calidoustus* (AS3.15302): A–B. colonies on CYA, CY20S at 25 °C after 14 days; C–D. conidiophores; E. conidia. Bar = 10 μm

× 5–7 μm; vesicles subglobose, light brown, 9–18(–24) μm, biseriate; metulae very short, 3.5 × 2–3 μm; phialides ampuliform, 7–7.5 × 2–3 μm, with short collula; conidia spheroidal, conspicuously echinulate, 2.5–3.6(–4) μm; Hülle cells not found.

Colonies on CY20S at 25 °C growing rapidly, attaining 46–49 mm in diam. in 14 days, moderately deep, depressed at centers, margins entire, floccose; conidiogenesis heavy, Drab Gray to Light Drab; mycelia white; exudate and soluble pigment absent; reverse Pale Yellow-Green.

Colonies on CYA at 37 °C after 14 days: growing fast, 48–50 mm in diam., plane, centrally umbonate, margins entire, compactly floccose; conidiogenesis heavy, Drab Gray; mycelia white, only conspicuous at margins; exudate and soluble pigment absent; reverse Pale Yellow-Green to Light Viridine Green.

ISOLATE EXAMINED: China, Shandong Province, foot of Mount Tai, from soil, 23 July 2011, Z. Yang (CGMCC AS3.15302).

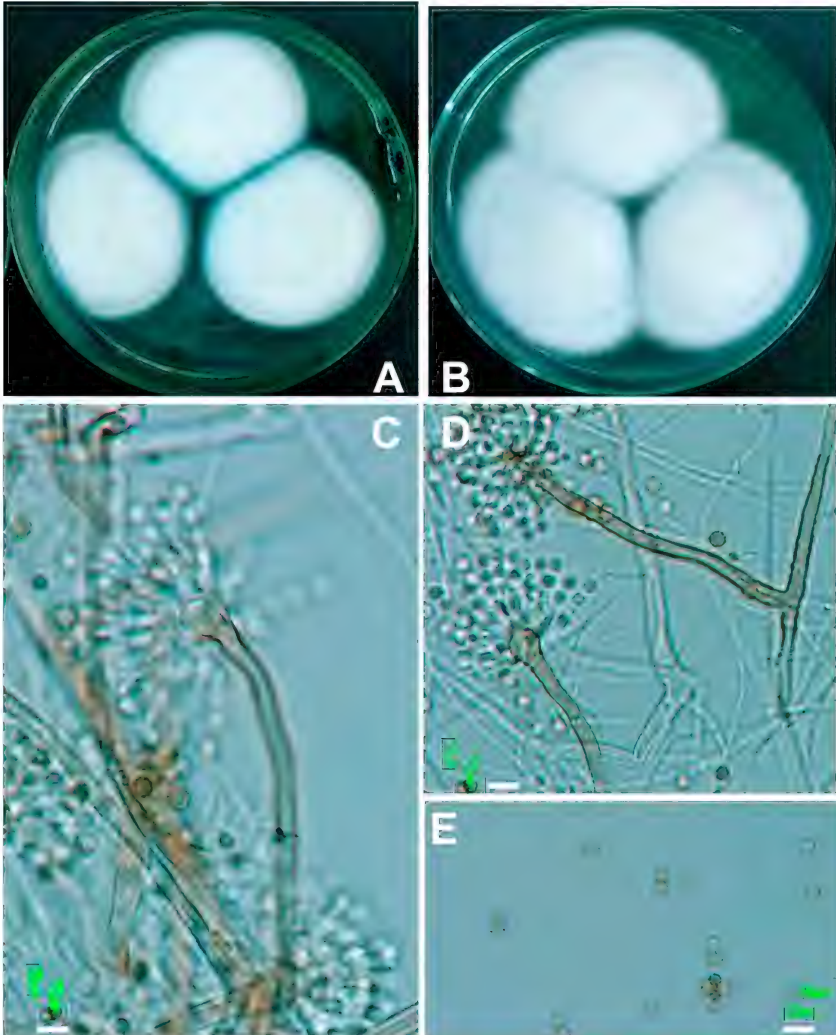


PLATE 3. *Aspergillus keveii* (AS3.15305): A–B. colonies on CYA, CY20S at 25 °C after 14 days; C–D. conidiophores; E. conidia. Bar = 10 µm

Aspergillus keveii Varga, Houbraken & Samson

PL. 3

Colonies on CA at 25 °C growing moderately, reaching 38–40 mm in diam. in 14 days, flat, sparse, floccose, margins fimbriate; conidiogenesis sparse; mycelia white; exudate and soluble pigment absent; reverse white.

Colonies on CYA at 25 °C growing rapidly, reaching 44–47 mm in diam. in 14 days, deep, margins fimbriate, floccose; conidiogenesis sparse, mostly in the central areas, Light Drab; mycelia white with Pale Brownish Vinaceous tinge; exudate and soluble pigment absent; reverse Pale Ochraceous-Buff.

Conidial heads small, radiate; conidiophores borne from aerial hyphae, stipes smooth-walled, brown in color, 200–280 × 5–7.5 µm; vesicles subglobose, brown-colored, 10–18 µm in diam., biserial; metulae short, 3.5 × 2–3 µm; phialides ampuliform, 7–7.5 × 2–3 µm, with short collula; conidia spheroidal, conspicuously roughened, 3–3.6(–4) µm; Hülle cells not observed.

Colonies on CY20S at 25 °C growing moderately, reaching 35–38 mm in diam. in 14 days, deep, margins fimbriate, floccose; conidiogenesis sparse; mycelia white with Pale Grayish Vinaceous tinge; exudate and soluble pigment absent; reverse Light Ochraceous-Salmon.

Colonies on CYA at 37 °C showing no growth after 14 days.

ISOLATE EXAMINED: China, Shandong Province, foot of Mount Tai, from soil, 23 July 2011, Z. Yang (CGMCC AS3.15305).

Aspergillus pseudodeflectus Samson & Mouch.

PL. 4

Colonies on CA at 25 °C growing moderately, attaining 28–30 mm in diam. in 14 days, deep, flat, floccose; conidiogenesis absent; mycelia white; exudate and soluble pigment absent; reverse white.

Colonies on CYA at 25 °C fast-growing, reaching 43–45 mm in diam. in 14 days, deep, convex, floccose; conidiogenesis moderate, Light Drab; mycelia white; exudate and soluble pigment absent; reverse Marguerite Yellow to Reed Yellow.

Conidial heads small, radiate; conidiophores borne on aerial hyphae, stipes curved and slender, smooth-walled but with occasional concretions, light brown in color, (50–)100–180 × 2–3.5 µm; vesicles hemispherical, brown-colored, 4–7.5 µm in diam., biserial; metulae very short, 3.5 × 2–3 µm; phialides ampuliform, 7–7.5 × 2–3 µm, with long collula up to 3.5 µm; conidia spheroidal, warty, 2.5–3.6(–4) µm; Hülle cells not observed.

Colonies on CY20S at 25 °C growing rapidly, reaching 42–43 mm in diam. in 14 days, deep, convex with depression at centers, floccose; conidiogenesis moderate, Light Drab to Drab Gray; mycelia white; exudate and soluble pigment absent; reverse Reed Yellow.

Colonies on CYA at 37 °C growing rapidly, reaching 48–50 mm in diam. in 14 days, flat, annularly and radiately plicate, umbonate lightly in centers, floccose;



PLATE 4. *Aspergillus pseudodeflectus* (AS3.15306): A–B. colonies on CYA, CY20S at 25 °C after 14 days; C–E. conidiophores; F. conidia. Bar = 10 μ m

conidiogenesis heavy, Light Cinnamon-Drab to Light Drab; mycelia white; exudate and soluble pigment absent; reverse Dark Olive to Buffy Brown.

ISOLATE EXAMINED: China, Shandong Province, foot of Mount Tai, from soil, 23 July 2011, Z. Yang (CGMCC AS3.1506–3.15310).

Discussion

Emericella heterothallica was the first reported heterothallic teleomorph associated with the aspergilli. Raper & Fennell (1965) reported the production of cleistothecia following the cross of yellow series and reddish orange series. Houbraeken et al. (2007), however, were unable to reproduce this result.

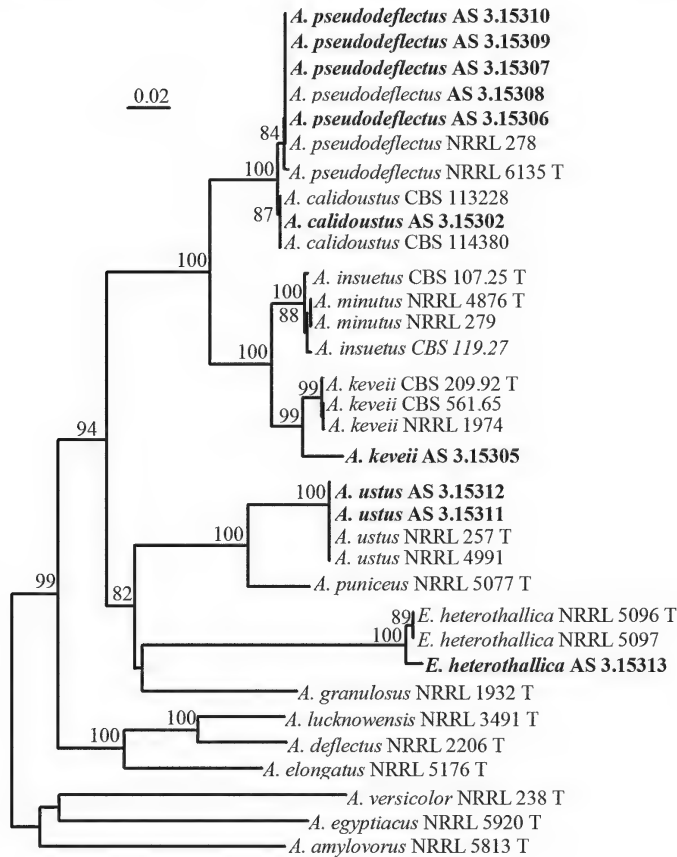


PLATE 5. The NJ tree inferred from the partial sequences of calmodulin gene data set. Bootstrap percentages > 70% derived from 1000 replicates are indicated at the nodes. Bar = 0.02 substitutions per nucleotide position.

According to the characters presented, isolate AS3.15313 is a member of the reddish orange series. Although *E. heterothallica* has only been reported rarely, our observation of this species in China suggests that, despite its relative rarity, it is geographically widely distributed.

Raper & Fennell (1965) cited six isolates —WB275 (type culture), WB278, WB280, WB281, WB1974, WB4876— as typical of the six morphotypes they recognized from among the hundreds of *A. ustus* isolates they had examined. It is interesting how predictive of distinct species their observations were.

Based on partial β -tubulin, calmodulin, actin genes, and ITS2-5.8S-ITS2 rDNA sequences, Houbraken et al. (2007) restricted *A. ustus* to isolates WB275 and WB280 and designated WB1974 as a new species, *A. keveii*. Varga et al. (2008) included WB281 in their new taxon, *A. calidoustus*. We observed much variation among our isolates under the name of *A. ustus* sensu Raper & Fennell (1965), with prominent differences in the stipe and vesicle shapes and dimensions. The growth rate at 37 °C varied greatly, some strains rapid, others moderate, still others with no growth at all. Conidiation was also highly variable, ranging from abundant to none depending on the isolates and culture conditions, and conidial color ranged from grey-green to drab and brown. These morphological differences suggest that multiple cryptic taxa may remain embedded in *A. ustus* sensu Raper & Fennell (1965). Phylogenetic analyses of three genetic markers (the *nuC* ITS1-5.8S-ITS2 rRNA region and the partial β -tubulin and calmodulin gene sequences) affiliate our isolates with the four taxa included in sect. *Usti* by Houbraken et al. (2007) and Samson et al. (2011).

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**Systematics of the *Gomphales*:
the genus *Gomphus* sensu stricto**ADMIR J. GIACHINI^{1*}, CARLA M. CAMELINI¹, MÁRCIO J. ROSSI¹,
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ABSTRACT — *Gomphus* sensu lato (*Gomphales*) was described to include species of cantharelloid-gomphoid fungi that had ‘meruloid’ (wrinkled) hymenia and verrucose spores. *Gomphus* sensu stricto is currently characterized by unipileate to merismatoid (composed of several pilei) basidiomata, depressed funnel- to fan-shaped pilei, presence of clamp connections, and verrucose spores; it has three described species: *G. brunneus*, *G. clavatus*, and *G. crassipes*. Basidiomata and spore features are reliable identifiers of *Gomphus* sensu stricto that distinguishes the species from other genera in the *Gomphales*.

KEY WORDS — *Gloeocantharellus*, *Phaeoclavulina*, *Turbinellus*

Introduction

The genus *Gomphus* was originally proposed as a segregate from the genera *Clavaria*, *Geoglossum*, *Mitrula*, and *Spathularia* and described as “thickened, truncate, smooth, laterally plicate-venose, the pileus weakly developed” (Persoon 1797). No species were assigned to the genus when described. The first citation of a species for *Gomphus* came only when Gray (1821) described *G. clavatus* based on *Merulius clavatus* Pers., a species known to have merismatoid (composed of several pilei) basidiomata, orangish brown to violet pilei, violet hymenia, and verrucose non-anastomosed ornamented spores.

From the earliest citations of Persoon (1797) to the latest descriptions of Petersen (1971), the systematics and nomenclature of *Gomphus* have had a checkered history. Before Giachini (2004) analyzed the molecular phylogeny of 320+ collections of *Gomphus* sensu lato and related genera in the *Gomphales* and reviewed the systematics and nomenclatural history for this group, species of *Gomphus* sensu stricto were assigned to three different genera: *Cantharellus*

(Fries 1821), *Craterellus* (Fries 1838), and *Neurophyllum* (Doassans & Patouillard 1886).

The reduced number of distinctive morphological features and (more importantly) the lack of molecular data to clarify the systematics of this group have contributed to its confused classification. Nevertheless, the significant contributions by many taxonomists have led to a better understanding of the overall placement of gomphoid fungi.

Recently Giachini & Castellano (2011) presented a new classification for *Gomphus* sensu lato. Giachini (2004) and Giachini & Castellano (2011) emphasized that *Gomphus* sensu stricto is the only genus in the *Gomphaceae* with strictly violet, lavender-brown, or milky-coffee colored hymenia, distinguishing it from other representatives of *Gomphus* sensu lato (*Gloeocantharellus*, *Phaeoclavulina*, *Turbinellus*) characterized by orange, brown or greenish olive hymenia. Furthermore, all *Gomphus* species produce clamp connections and verrucose spores. The unique combination of these morphological characteristics separates *Gomphus* from other genera within the *Gomphales*.

Below we review the important diagnostic characters of *Gomphus* sensu stricto and provide a dichotomous key and complete descriptions of the three currently accepted species: *G. brunneus*, *G. clavatus*, and *G. crassipes*. For discussion on the nomenclatural history of *Gomphus* sensu lato see Giachini (2004), Giachini et al. (2010), and Giachini & Castellano (2011).

Materials & methods

Collections examined

Collections of *Gomphus* were obtained from BR, DAOM, FH, K, MICH, NYS, OSC, S, SFSU, TENN, and WTU (<http://www.nybg.org/bsci/ih/ih.html>). Since Dufour (1889) did not assign a type for *G. crassipes* and the only known collections (two) deposited at the herbarium RAB were not granted for loan, its description and further notes were taken from the relevant literature.

In addition to dried herbarium material, we examined fresh specimens of *G. clavatus* from northern California, Oregon, and Washington. These were collected mostly in late September to mid-December, the usual fruiting season. Therefore, colors for *G. clavatus* are based on fresh and dried material while for *G. brunneus* are based on dried material only.

After selected collections were photographed and macroscopic features recorded, they were dried within 24 h of collection with a forced-air food dehydrator set at 38–40°C, and deposited at OSC.

Habitat and fruiting patterns are based on information from the original descriptions and/or field observations.

Macroscopic characterization

Macroscopic features are based on original descriptions and herbarium notes supplemented by our own data on fresh or dried specimens. Features of the pileus,

hymenium, stipe, flesh, odor and taste were recorded whenever possible. Size of basidiomata may vary depending on conditions of the fruiting season, so the ranges of minimum and maximum sizes are presented as the range for 5–10 collections. Several specimens were cut longitudinally through the vertical axis to examine the context structure of pileus and stipe. Application of chemicals often used and referred to in the literature (Corner 1966, Petersen 1971) in describing species of *Gomphus sensu lato* proved of little value and so are not reported here.

Microscopic characterization

Features observed with a compound microscope were described from free-hand sections mounted in Melzer's reagent, 5% KOH, cotton blue, or H₂O. Structures were measured with an optical micrometer at $\times 1000$ magnification, mostly in mountants. Hyphal wall thicknesses are recorded as 'thin' $\leq 0.5 \mu\text{m}$ and 'thick' $> 0.5 \mu\text{m}$. The pileipellis and stipitipellis were examined in surface view. When material was available, dimensions were recorded from at least 3 specimens from each of at least 5 collections. Both mature and immature specimens were examined for developmental changes. Maturity was judged by the relative abundance of fully ornamented spores.

Spore shape was determined by the length-width ratio of 20–30 randomly selected spores (Kirk et al. 2008). Dimensions are given as: minlength–maxlength $\times$ minwidth–maxwidth, excluding ornamentation and apiculus. Immature or oversized spores were not included in the measurements, though variation was noted.

Taxonomy

Species are presented in alphabetical order. Descriptions include citation of all examined material and the herbarium where the type and other collections are deposited. Descriptions are based on notes available with collections, the observations made in this study, and literature reports.

Gomphus Pers., Tent. Disp. Meth. Fung.: 74 (1797) [non *Gomphus* (Fr.) Weinm. 1826].

(For synonymy, see Giachini & Castellano 2011:186.)

TYPE SPECIES: *Gomphus clavatus* (Pers.) Gray 1821.

BASIDIOMATA ≤ 18 cm tall, erect, unipileate to merismatoid, when merismatoid with two to several pilei arising from a single stipe. PILEUS 4–18 cm wide, plane to depressed, fan- to funnel-shaped, flexuous, fleshy, dry, glabrous (*G. brunneus*) to subpruinose, covered with brown hyphae that form minute separate and distinct patches toward the margin but merge into a continuous felty tomentum over the disc (*G. clavatus*), rosaceous, sordid yellow to orange-brown, creamy violet, violet, brown; margin lacerate to crenate. HYMENIUM decurrent, wrinkled, generally longitudinally oriented, dichotomous, often to rarely anastomosing, reticulate to almost poroid (*G. brunneus*), especially near the stipe apex, more lamellate toward the margin of the pileus, violet, vinaceous brown to milky-coffee colored. STIPE ≤ 80 mm long, solid, cylindrical to tapering downward, generally dilating into the pileus, pale violet to brown, nearly black in *G. crassipes*, often pale red-brown when handled. CONTEXT

firm, pliable, often with anastomosing cavities, especially in *G. clavatus*, white, off-white, violet, pale rose, brownish pink on exposure. ODOR faint to sweet. TASTE mild to bitter. SPORE PRINT brown for *G. brunneus* and *G. clavatus*, not recorded for *G. crassipes*.

PILEIPELLIS of scattered to fasciculate, simple or branched, rarely slightly inflated hyphae, in *G. clavatus* with pileocystidia; clamp connections present. STIPITIPELLIS of parallel hyphae at surface, interwoven beneath, hyaline, $\leq 2\ \mu\text{m}$ wide; clamp connections present. PILEUS and STIPE CONTEXT of interwoven, hyaline hyphae 2.5–6 μm wide, generally uninflated except adjacent to the clamp connections. HYMENIAL TRAMA of thin- to thick-walled hyphae; clamp connections present. SUBHYMENIAL TRAMA of interwoven, hyaline hyphae $\leq 8\ \mu\text{m}$ wide; clamp connections present. BASIDIA $\leq 100\ \mu\text{m}$ long, 12 μm wide, clavate, in general with (2–)4 slightly divergent, slightly incurved sterigmata; clamp connections present. HYMENIAL CYSTIDIA absent. BASIDIOSPORES 7.5–17 $\times$ 3.5–7.5 μm , orange, yellow-brown to dark olive in mass, ellipsoid to obovoid; ornamentation verrucose, cyanophilic; apiculus eccentric (not recorded for *G. crassipes*).

ECOLOGY & DISTRIBUTION: epigeous, solitary to gregarious or caespitose, in leaf litter or terrestrial. When gregarious, basidiomata are often separated by a few centimeters. Encountered in Africa, Asia, Europe, and North America.

REMARKS: Defining boundaries for species within *Gomphus* sensu lato has been a constant challenge because of the lack of consistent morphological features. Only recently have morphology and molecular techniques combined to provide a natural classification for the *Gomphaceae*. Giachini et al. (2010) and Giachini & Castellano (2011) verified by molecular and morphological data that species of *Gomphus* sensu stricto (*G. brunneus*, *G. clavatus*, and *G. crassipes*) are in a lineage separate from all other species of *Gomphus* sensu lato. Accordingly, *Gomphus* sensu stricto is reduced to three species vs. the 35 formerly assigned to *Gomphus* sensu lato. Main features diagnosing *Gomphus* are the smooth to subpruinose pileus, wrinkled or poroid-like hymenium, and clamp connections. Species of *Gomphus* sensu stricto occur across the northern hemisphere and as far south in Africa as the Democratic Republic of the Congo.

Gomphus brunneus (Heinem.) Corner, Ann. Bot. Mem. 2: 116. 1966.

FIG. 1

(For synonymy, see Giachini & Castellano 2011:186.)

TYPE: DEMOCRATIC REPUBLIC OF CONGO. Equateur, Binga, April 1928, *M. Goosens-Fontana* 683 (BR A253).

BASIDIOMATA $\leq 9 \times 4\ \text{cm}$, unipileate, erect, clavate, truncate at apex. PILEUS $\leq 4\ \text{cm}$ wide, depressed to funnel-shaped, thick in the center, thin toward the ascending and somewhat lacerate margin, brown or occasionally more or less rosaceous, with paler tones in the center. HYMENIUM wrinkled, reticulate

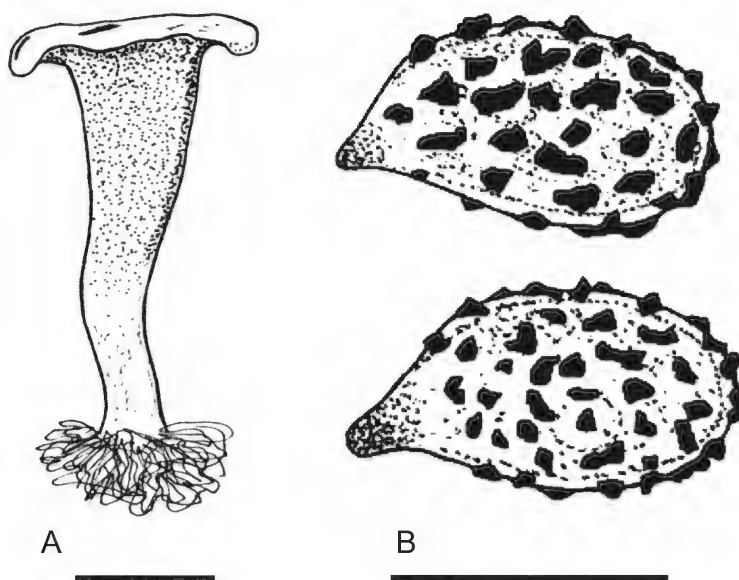


FIG. 1. *Gomphus brunneus*. A. Basidioma (bar = 2 cm). B. Basidiospores (bar = 5 μ m).

to almost poroid, pale violet to milky-coffee colored. STIPE ≤ 80 mm long, tapered downward, dilating obconically into the pileus, brown, the base white tomentose. CONTEXT pliable, white, then brownish pink on exposure. ODOR not recorded. TASTE bitter. SPORE PRINT brown.

PILEIPELLIS of scattered to fasciculate, simple hyphae; clamp connections present. STIPITIPPELLIS of hyaline hyphae ≤ 2 μ m wide; clamp connections present. PILEUS and STIPE CONTEXT of interwoven, hyaline hyphae 2.5–6 μ m wide; clamp connections present. HYMENIAL TRAMA of thin-walled hyphae; clamp connections present. SUBHYMENIAL TRAMA of slightly thickened, hyaline hyphae 3–7 μ m wide, with refractive walls; clamp connections present. BASIDIA 50 $\times$ 8 μ m, with 2–4 sterigmata. HYMENIAL CYSTIDIA absent. BASIDIOSPORES 7.5–10 $\times$ 3.5–5 μ m, yellow-brown in mass, ellipsoid to obovoid; ornamentation of cyanophilic warts arranged more or less in longitudinal rows; apiculus rounded, eccentric.

ECOLOGY & DISTRIBUTION terrestrial, in leaf litter, on floor of dry forests of Cameroon (Roberts 1999), the Democratic Republic of Congo, and Uganda (Corner 1966, Roberts 1999).

ADDITIONAL SPECIMENS EXAMINED: UGANDA: Gunda Forest, May 1918, T.D. Maitland 275 (as *Gomphus clavatus* var. *parvisporus*; K 57307).

REMARKS: *Gomphus brunneus* is distinguished by its simple basidiomata with smooth pilei, weakly wrinkled hymenia, clamped hyphae, and comparatively small basidiospores. Its morphology, distribution, and molecular profile make it unique (Giachini 2004, Giachini et al. 2010, Giachini & Castellano 2011).

Only two *G. brunneus* herbarium collections were available for examination. After studying the type, Roberts (1999) considered *G. clavatus* var. *parvisporus* from Uganda (described by Corner 1966 and said to occur in Europe and the western USA) a synonym of *G. brunneus*. Both species are macroscopically similar with unusually small verrucose spores and are known only from equatorial Africa. Heinemann (1958, 1959) reported two collections from forests of the Democratic Republic of Congo, Africa. Corner (1966) reported one collection (as *Gomphus clavatus* var. *parvisporus*) from Cameroon, while Roberts (1999) cited two collections from Cameroon obtained in 1996 and reported abundant basidiomata from the same location on the following year. Otherwise, the species appears to be restricted to the Democratic Republic of Congo and Uganda.

For several decades *Cantharellus pseudoclavatus* A.H. Sm. (Smith & Morse 1947) was classified under *Gomphus* sensu lato (Corner 1966). After analyzing the type collection (MICH 6916), however, Giachini (2004) concluded that it better fit *Cantharellus*. The main morphological character justifying its removal from *Gomphus* sensu lato was the presence of 8-spored basidia, a feature not found elsewhere in *Gomphus* but present in *Cantharellus*. Petersen (1971) had previously transferred the species to *Pseudocraterellus*, a genus which also lacks 8-spored sterigmata. Although Corner (1966) suggested *Gomphus clavatus* var. *parvisporus* occurs in Europe and is common in western USA, he mentioned only specimens obtained in Africa in his original description of that variety. *Cantharellus pseudoclavatus* is rare under conifers of the western USA and is definitely distinct from *G. brunneus*.

Gomphus clavatus (Pers.) Gray, Nat. Arr. Brit. Pl. 1: 638. 1821.

FIG. 2

(For synonymy, see Giachini & Castellano 2011:187.)

BASIDIOMATA ≤17 cm tall, unipileate at first and then merismatoid with ≤15 subpilei. PILEUS ≤15 cm wide, the surface flat, subundulate, fan-shaped, glabrous to covered with brown hyphae that form minute separate and distinct patches toward the margin but merge into a continuous felty tomentum over the disc, orangish brown to overall creamy violet to dark violet; margin crenate. HYMENIUM surface wrinkled, generally longitudinally oriented, with or without discrete folds or pits, bright violet at the margin and junction with stipe and overall when immature, at maturity covered with spores and then paler (vinaceous brown). STIPE tomentose to hispid toward the base, glabrous above and there blending to pale violet, white at the base and where covered



FIG. 2. *Gomphus clavatus*. A–B. Basidiomata. C. Spores (bar = 10 μ m).

(soil, debris), often pale red-brown where handled. CONTEXT often with anastomosing cavities, violet around those, off-white to pale rose elsewhere. ODOR faint or none. TASTE mild. SPORE PRINT brown.

PILEIPELLIS often crowded, thin-walled, scattered to fasciculate, with simple or branched hyphae, usually incrustated apically, with pileocystidia 3–4.5 μm wide, rarely slightly inflated, $\leq 5.5 \mu\text{m}$, protruding 50–120 μm from the surface; clamp connections present. Hyphae and bases of pileocystidia often with brown amorphous deposits. STIPITPELLIS of periclinal and parallel, hyaline hyphae 1.5–2 μm wide at the surface, becoming interwoven beneath, with few projecting hyphae where stipe is glabrous, or fascicles of crowded hyphae where stipe is hispid; clamp connections present. PILEUS and STIPE CONTEXT of thin- to slightly thick-walled, generally oriented on the long axis of the basidioma but profusely interwoven, hyaline hyphae 2.5–6 μm wide, generally uninflated except adjacent to the clamp connections, where it is slightly thick-walled ($\leq 1 \mu\text{m}$ thick) and $\leq 12 \mu\text{m}$ wide. HYMENIAL TRAMA hyphae more loosely interwoven than the rest of the context, undifferentiated from the SUBHYMENIAL TRAMA. BASIDIA 60–95 $\times$ 8.5–12 μm , clavate, collapsing after spore discharge, hyaline, with (2–)4 slightly divergent, slightly incurved sterigmata $\leq 10 \mu\text{m}$ long; clamp connections present at base. HYMENIAL CYSTIDIA not observed. BASIDIOSPORES (9–)10–15(–17) $\times$ 4–7.5 μm , orange in Melzer's reagent, dark olive in mass (KOH), ellipsoid to obovoid, contents weakly cyanophilic; ornamentation of cyanophilic warts, usually in discrete, raised patches that give an undulate appearance to the spore surface; apiculus prominent, eccentric.

ECOLOGY & DISTRIBUTION: solitary, gregarious to caespitose, when gregarious often separated by a few centimeters, terrestrial. Generally in conifer forests and suggested to form mycorrhizae with species of *Abies* (Pantidou 1980) and *Picea* (Agerer et al. 1998). Known from Austria (Petersen 1971), Canada (East and West, Petersen 1971), China (Corner 1966), Czech Republic (Kluzák 1994), France (Doassans & Patouillard 1886), Greece (Petersen 1971), Italy (Petersen 1971), Japan (Corner 1966), Lithuania (Urbonas et al. 1990), Mexico (Petersen 1971), Pakistan (Corner 1966), Poland (Adamczyk 1996), Russia (Bulakh 1978, Bulakh & Govorova 2000), Spain (Fernando Sanchez, pers. comm., 10 Oct. 1999), Sweden, Switzerland (Petersen 1971), Turkey (Sesli 1997), and the USA (especially northwestern/northeastern and into the Appalachian Mountains). Loans were requested from herbaria located in most of these countries but several did not respond, so our examination of specimens is restricted to those listed under collections examined.

ADDITIONAL SPECIMENS EXAMINED: CANADA. BRITISH COLUMBIA, Vancouver Island, 15 October 1962, *M. Pantidou* (DAOM 91369). Quebec, Duchesnay, 26 Aug. 1938, *H.S. Jackson* (DAOM 8624). SWEDEN: Uppsala, Södermanland, Handen, 4 Sep. 1998, *L. Kerwién* (S); Fiby Urskog, 20 Sep. 1998, *A. Taylor* (S). UNITED STATES. CALIFORNIA, DEL NORTE CO., Del Norte Coast Redwoods State Park, 9 Dec. 1999, *M.A.*

Castellano (OSC 97685); Jedediah Smith Redwoods State Park, 17 Nov. 1961, *D. Isely* 1479 (WTU), and 12 Nov. 1966, *H.D. Thiers* (SFSU 17740); Siskiyou Nat. Forest, Smith River, 30 Nov. 1937, *A.H. Smith* 9243 (MICH); EL DORADO CO., Pleasant Valley, 22 October 1967, *D.E. Stuntz* 14410 (WTU); MENDOCINO CO., 5 Nov. 1961, *D. Largent* 71 (SFSU); Aleuria Glenn, 14 Nov. 1967, *R.H. Petersen* (TENN 33256); Aleuria Glenn, along Rd. 404, 25 October 1986, *M. Seidl* 2120 (WTU); Jackson State Forest, 5 Nov. 1967, *R.H. Petersen and H.D. Thiers* (TENN 33174), and 8 Dec. 1999, *A.J. Giachini, M.A. Castellano and E. Nouhra* (OSC 97679, 97680, 97681, 97682, 97683, 97684). IDAHO, BONNER CO., Priest River, Binarch Creek, 9 Sep. 1966, *R.H. Petersen* (TENN 32166); Priest River Experimental Forest, Canyon Creek Road, 11 Nov. 1939, *A.H. Slipp* 621 (MICH). MASSACHUSETTS, ESSEX CO., Gloucester, Aug. 1877, *unnamed collector* (FH). NEW YORK, SARATOGA CO., Ballston Lake, Branch, 1879, *C.H. Peck* (holotype of *Cantharellus brevipes*; NYS). OREGON, BENTON CO., Siuslaw Nat. Forest, Mary's River, 24 October 1999, *S. Ashkannejhad* (OSC 97622); CLACKAMAS CO., Wemme, 23 Sep. 1946, *A.H. Smith* 23644 (MICH); Coos Co., South Slough, 23 Sep. 1985, *C. Ardrey* 800 (WTU), and 3 Sep. 1992, *C. Ardrey* 1806 (WTU); LANE CO., Cougar Reservoir, Road 19, 27 Nov. 1999, *A.J. Giachini* (OSC 97671, 97672, 97673, 97674, 97675, 97676, 97677); Eugene, 30 October 1999, *unnamed collector* (OSC 97646); H. J. Andrews Experimental Forest, Lookout Mt., Rd. 1506, 23 October 1999, *A.J. Giachini* (OSC 97616, 97620, 97669, 97670, 97678); H. J. Andrews Experimental Forest, 23 October 1999, *A.J. Giachini* (OSC 97617), 5 Nov. 1999, *A.J. Giachini* (OSC 97656), and 7 Nov. 2000, *Y. Yano* (OSC 97694); Oakridge, Road 1934, 15 Nov. 1999, *A.J. Giachini* (OSC 97668); Willamette Nat. Forest, 25 October 1999, *C. Lefevre* (OSC 97623); LINN CO., Sweet Home, 4 Nov. 2000, *A.J. Giachini* (OSC 97696, 97698); Willamette Nat. Forest, Pamela Lake Trail head, 2 Nov. 1999, *A.J. Giachini* (OSC 97624); MARION CO., Detroit Lake, Road 46, 15 Nov. 1999, *A.J. Giachini* (OSC 97667). WASHINGTON, CHELAN CO., Lake Wenatchee, 7 Nov. 1980, *D.E. Stuntz* 21131 (WTU); CLALLAM CO., Morse Creek camp, 17 October 1942, *D.E. Stuntz* 1245 (WTU); Olympic Mountains, 30 October 1931, *H.S. Hotson* (WTU); KINGS CO., Snoqualmie Nat. Forest, Asahel Curtis Nature Trail, 4 Aug. 1992, *G.R. Walker* 10600 (WTU); LEWIS CO., Mt. Rainier Nat. Park, Bumping Lake, October 1937, *D.E. Stuntz* 759 (WTU); PIERCE CO., Mt. Rainier Nat. Park, Lower Tahoma Lake, 23 Sep. 1948, *D.E. Stuntz* 4732 (WTU); Old Tahoma campground, 23 Sep. 1960, *D.E. Stuntz* 12000 (WTU); Mt. Rainier National Park, Tahoma Creek, 18 Aug. 1948, *D.E. Stuntz and A.H. Smith* 4003 (WTU); Mt. Rainier Nat. Park, Tahoma Creek, 23 Sep. 1960, *D. Isely* 1257 (WTU).

REMARKS: *Gomphus clavatus* has traditionally been placed in a monotypic subgenus (*Gomphus* subg. *Gomphus*) based on morphological features (Corner 1966). However, molecular studies by Giachini (2004) and Giachini et al. (2010) support its placement together with *G. brunneus* and *G. crassipes* in *Gomphus sensu stricto* and suggest it to be phylogenetically closely related to species of *Gloeocantharellus*. Petersen (1971) noted that these two genera share several morphological similarities, the most striking of which is that both exhibit the same reaction to 10% KOH applied to the basidioma pileus. In reference to *G. clavatus* Petersen (1971) stated, "the normal color immediately becomes a most unique salmon-yellow, almost precisely the normal color of the pileus and stipe of *Gloeocantharellus purpurascens* (Hesler) Singer." The pileus surface tomentum of *G. clavatus* is composed of pileocystidia, just as in *G. purpurascens*,

and, even though the pileocystidia of the two species differ in shape and orientation, they have a similar brown agglutinating substance. Despite these similarities, the molecular evidence shows *Gomphus* and *Gloeocantharellus* to be in distinctly different, but related lineages (Giachini et al. 2010).

Other taxa in the *Gomphaceae* share the merismatoid habit of *G. clavatus*, notably *Phaeoclavulina grandis* (Corner) Giachini, *P. guadelupensis* (Pat.) Giachini, *P. subclaviformis* (Berk.) Giachini, and *P. viridis* (Pat.) Giachini and *Gloeocantharellus dingleyae* (Segedin) Giachini, *G. novae-zelandiae* (Segedin) Giachini, and *G. pallidus* (Yasuda) Giachini.

Gomphus crassipes (L.M. Dufour) Maire, in Maire & Werner, Mém. Soc. Sci. Nat.

Maroc 45: 81 (1937).

FIG. 3

(For synonymy, see Giachini & Castellano 2011:187.)

Lectotype designated here, Pl. 13, in L.M. Dufour, Rev. Gen. Bot. 1: 357–358. 1889.

BASIDIOMATA ≤18 cm tall, unipileate or occasionally merismatoid. PILEUS 4–18 cm wide, plane to slightly depressed in the center, funnel-shaped to fan-shaped, non hygrophanous, sordid yellow to orangish brown; margin subundulate. HYMENIUM decurrent, wrinkled, occasionally anastomosing, dichotomous, violet. STIPE 40–70 × 25–45 mm, cylindrical to slightly tapering downward, almost entirely underground, central or lateral, nearly black. CONTEXT firm, pale violet, marbled in the stipe with violet tones. ODOR sweet. TASTE not recorded. SPORE PRINT color not recorded.

All HYPHAE 5–8 μm wide; clamp connections present. BASIDIA 70–100 × 8–10(–12) μm, clavate, with (2–)4 straight sterigmata. HYMENIAL CYSTIDIA absent. BASIDIOSPORES (11–)13–15(–17) × 5.5–6(–7) μm, pale orange in mass, ellipsoid to obovoid; ornamentation of cyanophilic, fine warts; data on apiculus not recorded (Dufour 1889, Maire 1914).

ECOLOGY & DISTRIBUTION: on the ground, generally grouped, in conifer forests in Algeria (Dufour 1889, Maire 1914, Maire & Werner 1937), Morocco (Malençon 1958), and Spain (Miguel À. Pérez-De-Gregorio i Capella, pers. comm., 5 Jan 2004).

REMARKS: As the only known collections are at herbarium RAB in Morocco, and our loan request was denied due to the material's fragile condition, our description is based on Dufour (1889), Maire (1914), and Maire & Werner (1937). Dufour (1889), who did not cite any collection or herbarium for the type species, stated that “cette espèce est assez voisine du *Cantharellus brevipes* Peck.” As *C. brevipes* is a synonym of *G. clavatus*, in effect Dufour related *G. crassipes* to *G. clavatus*, with similar macro- and microscopic characteristics, differing only by the violet to marbled violet color of the context, which is violet, off-white to pale rose in *G. clavatus*. However, its overall spore size and

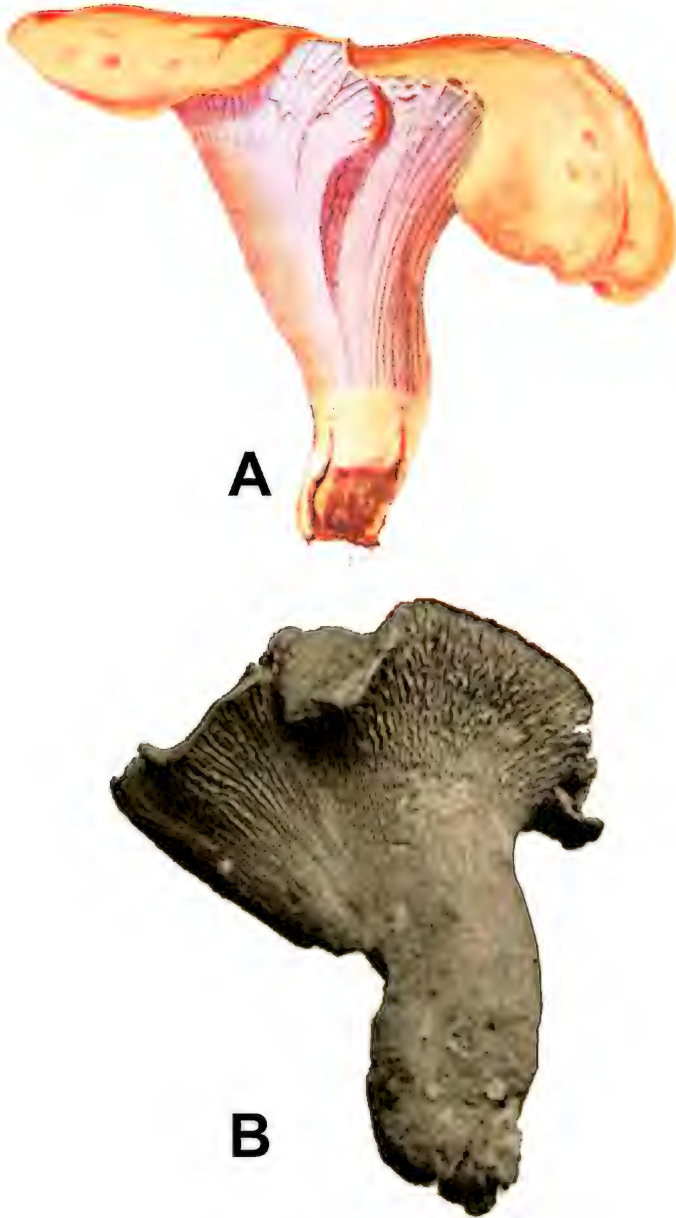


FIG. 3. *Gomphus crassipes*.

A. Basidiomata [from Maire (1914: Pl. VII.2)].

B. Dried basidiomata [modified from Dufour (1889; Pl. 13), with permission].

geographic distribution suggest that *G. crassipes* is distinct from *G. clavatus* (Giachini et al. 2010).

Only a few other references to *G. crassipes* are observed in the literature. Maire (1914), who transferred Dufour's *C. crassipes* to *Neurophyllum*, provided a detailed description and excellent drawing of its basidiomata and spores (Maire 1914) and subsequently (Maire & Werner 1937) transferred the species to *Gomphus*. Later Malençon (1958) called attention to the putative relationship of *G. crassipes* to the coral fungi, especially to *Ramaria*, but observed that Maire's drawings were not good representations of what Dufour (1889) had described as *C. crassipes* and that in particular the were not those suggested by Dufour. This species "pulls too much into the yellows and reds" according to Malençon (1958), who described *G. crassipes* as having a pale ochre to rose pileus surface. However, as seen for other species of *Gomphus* sensu lato, (e.g., *G. clavatus*) the color of both pileus and hymenium surface can vary considerably. For *G. clavatus*, for example, the pileus surface can be orangish brown to overall creamy violet to dark violet. It is possible that Maire (Maire & Werner 1937) observed a color variant of *G. crassipes*.

Because the designated lectotype does not adequately characterize the species, an epitype is desirable. However, we refrain from designating an epitype since no herbarium specimen is available and Malençon (1937) challenged the Maire's (1914) excellent illustration. A fresh collection (preferably from Morocco) that can provide DNA sequences would be an ideal candidate for epitypification.

Key to species

- 1a. Basidiomata initially unipileate, then becoming merismatoid with ≤ 15 subpilei; pileus fan-shaped, orangish brown, creamy violet to dark violet; hymenium wrinkled, violet, vinaceous brown, lavender-brown; Asia, Europe, North America *G. clavatus*
- 1b. Basidiomata unipileate to merismatoid; pileus depressed, funnel-shaped, occasionally fan-shaped, brown to rosaceous, sordid yellow to orangish brown; hymenium wrinkled, reticulate to almost poroid, violet to milky-coffee colored; Africa, Spain 2
- 2a. Spores $7.5\text{--}10 \times 3.5\text{--}5 \mu\text{m}$, verrucose; basidiomata unipileate; pileus brown or occasionally rosaceous; hymenium slightly wrinkled, reticulate to almost poroid, pale violet to milky-coffee colored; Cameroon, Democratic Republic of Congo, Uganda *G. brunneus*
- 2b. Spores $(11\text{--})13\text{--}15\text{--}(17) \times 5.5\text{--}6\text{--}(7) \mu\text{m}$, finely verrucose; basidiomata occasionally merismatoid; pileus sordid yellow to orangish brown; hymenium wrinkled, violet; Algeria, Morocco, and Spain *G. crassipes*

Discussion

Gomphus was originally described to include cantharelloid-gomphoid species that produced basidiomata resembling those of *Cantharellus*. The recent molecular analyses of Giachini et al. (2010), which sharpened generic and species concepts within the *Gomphaceae* and revealed *Gomphus* sensu lato as non-monophyletic, support redistribution of those into *Gomphus* sensu stricto, *Gloeocantharellus*, *Phaeoclavulina*, and *Turbinellus*. Only three original species are retained within *Gomphus* sensu stricto — *G. brunneus* characterized by somewhat funnel-shaped pilei and *G. clavatus* and *G. crassipes*, characterized by fan- to funnel-shaped pilei.

Gomphus clavatus, widely distributed in the northern hemisphere, is believed to form ectomycorrhizae with species of *Abies* (Pantidou 1980) and *Picea* (Agerer et al. 1998). It is the most easily identified of the three species, due primarily to the size and color of its basidiomata. Since the development and implementation of the Northwest Forest Plan in the United States (USDA/USDI 1994a,b, 2000, 2001), *G. clavatus* has been considered rare and potentially restricted to old-growth forests of the Pacific Northwest (PNW), requiring all federal and state land to be surveyed for the species before any management activity could be approved.

Both *G. brunneus* and *G. crassipes* are known only from a few collections from Africa and Spain, as noted above.

Morphologically *Gomphus* sensu stricto is readily separated from the other gomphalean genera based on the violet, violet-brown to orangish brown colors and fan- to somewhat funnel-shaped basidiomata pilei.

Species of *Gomphus* sensu stricto most resemble those of the resurrected genus *Phaeoclavulina* (Giachini & Castellano 2011), which share fan- to funnel shaped pilei and clamp connections. However, they differ based on spore ornamentation, with most *Phaeoclavulina* species diagnosed by echinulate, subreticulate, or reticulate spores in contrast to the strictly verrucose spores of *Gomphus*.

Even though all *Turbinellus* species were assigned to *Gomphus* until recently, they share only few morphological characters, most notably spore ornamentation. They differ in basidiomata shape, color, and presence of clamp connections (absent in *Turbinellus*).

Gomphus and *Gloeocantharellus* occupy an important position in the evolution of the fungi in the *Gomphales*. Molecular analyses (Giachini et al. 2001, Giachini et al. 2010, Humpert et al. 2001), indicate *Gloeocantharellus* and *Gomphus* as ancestral to all other genera within the *Gomphales*. The two genera share spore shape and ornamentation and (for some *Gloeocantharellus* species) clamp connections.

Unavailability of samples restricted more in-depth evolutionary inferences of *Gomphus* to other genera in the *Gomphales*. Additional sampling, especially

for *G. crassipes*, is necessary to determine whether subgeneric arrangements exist within *Gomphus* sensu stricto and to infer higher-level relationships to other members of the *Gomphales*. A complete discussion on the evolutionary relationships and implications of this taxonomic classification in relation to other members of the *Gomphales* can be found in Giachini (2004), Hosaka et al. (2006), and Giachini et al. (2010).

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MYCOTAXON

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April–June 2012

**Myxomycetes from China 15:
Arcyria galericulata sp. nov.**BO ZHANG¹, TIAN-HAO LI², QI WANG¹ & YU LI^{1*}¹Engineering Research Center of Chinese Ministry of Education for Edible and Medicinal Fungi &²Institute of Agricultural Modernization,

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ABSTRACT — A new species, *Arcyria galericulata*, is described and illustrated with scanning electron micrographs. This species is characterized by a helmet-like (galeiform) structure at the top of the sporocarp. Holotype and isotype specimens are deposited in the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU), Changchun, China.

KEY WORDS — SEM, taxonomy, *Trichiaceae*

Introduction

Arcyria is a common and important genus of *Trichiaceae*. Since Wiggers established the genus in 1780, more than 55 species have been reported (Kirk et al. 2008, Lado 2001, 2005–12), of which 29 species have been reported in China (Chen & Li 1999; Chou 1937; Li & Li 1989; Li et al. 1993; Liu et al. 2002; Wang & Li 1995, 1996, 2006). A new species found on bark surface of dead log in Jingyuetan National Forestry Park, Jilin province, China, in July, 2009 is described and illustrated below.

Materials & methods

The fruiting bodies and microscopic structures of the new species were examined by light and scanning electron microscopes (Martin & Alexopoulos 1969; Li et al. 1993) and compared with other morphologically similar *Arcyria* species. Permanent slides are mounted in Hoyer's medium (Martin & Alexopoulos 1969). Coloured slides were prepared according to Robbrecht (1974) by spreading capillitium in a drop of 94% alcohol, determining colour after one minute, and then mounting in Hoyer's. Colour terms are given according to Anonymous (1969). Voucher specimens are deposited in the Herbarium of Mycological Institute, Jilin Agricultural University (HMJAU).

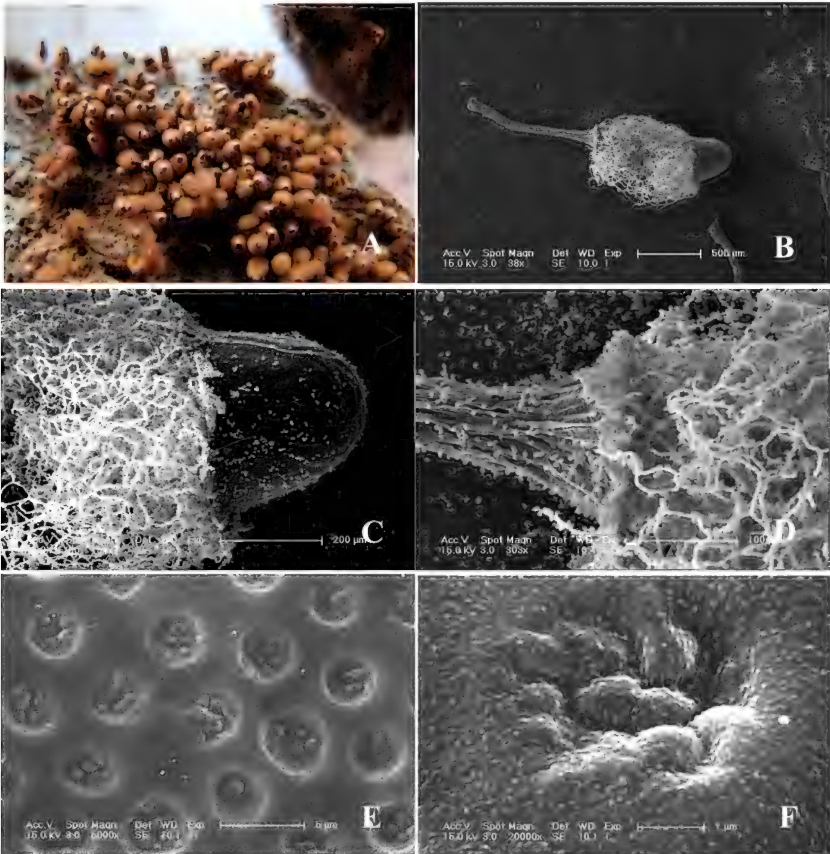


PLATE 1. *Arcyria galericulata* (Isotype): A, fruiting bodies. B, a complete, expanded sporocarp. C, galeiform structure at the top of the sporocarp and part of expansion of the capillitium. D, part of the stalk, showing longitudinal striations, and part of calyculus and sporocarp. E, outer surface of a galeiform structure marked with regular circular depressions. F, warty interior of a depression.

Taxonomy

Arcyria galericulata B. Zhang & Yu Li, sp. nov. PLATE 1–2
MYCOBANK MB564122

Differs from *Arcyria papilla* by its smaller sporocarp with a larger helmet-like protuberance and larger spores.

TYPE—China, Jilin province, Jingyuetan National Forestry Park, on the bark of a dead log, 26 July 2009, Zhang Bo 0628 (Holotype HMJAU10244; isotype HMJAU10245).

ETYMOLOGY—*galericulata* (Latin) = with a helmet-like covering, in reference to the galeiform protuberance at the top of the sporocarp.

SPOROCARPS aggregated or united in clusters of 3–8 (with fused stalks), stalked, erect, 1–1.5 mm in total height, 2–2.2 mm after expansion of the capillitium. Sporocarp obovoid or shortly cylindrical, fawn to snuff brown, fading to brown. HYPOTHALLUS common to a group of sporocarps. STALK 0.8–1.0 mm long, dark brown by transmitted light, filled with spore-like cells, cells 18–20 μm diam. at the base of stalk, subglobose. PERIDIUM single, membranous, persistent, with a helmet-like structure at the top of each sporocarp, outer surface of the structure marked with regular circular depressions with warted interiors. CALYCVLUS translucent, saucer-shaped, light, yellow to colourless by transmitted light, inner surface with many prominent warts, low irregular ridges forming a net with small protuberances. COLUMELLA absent. CAPILLITIUM tubular, elastic, branched and anastomosed, pale orange yellow by transmitted light, 3 μm diam. without ornamentations, 5–6 μm diam. with ornamentations, firmly attached to the calyculus, decorated with many cogs, half-rings and rings with irregular edges, sometimes with a faint reticulation. SPORES free, yellowish brown in mass, yellowish pale to colourless by transmitted light, 6–8 μm diam., densely warted, with scattered groups of more prominent warts.

COMMENTS: *Arcyria galericulata* has a galeiform structure at the top of the sporocarp. Only one other *Arcyria*, *A. papilla* Ejale & L.S. Gill (Ejale & Gill 1992), has a similar papillate structure at the top of the sporocarp but the

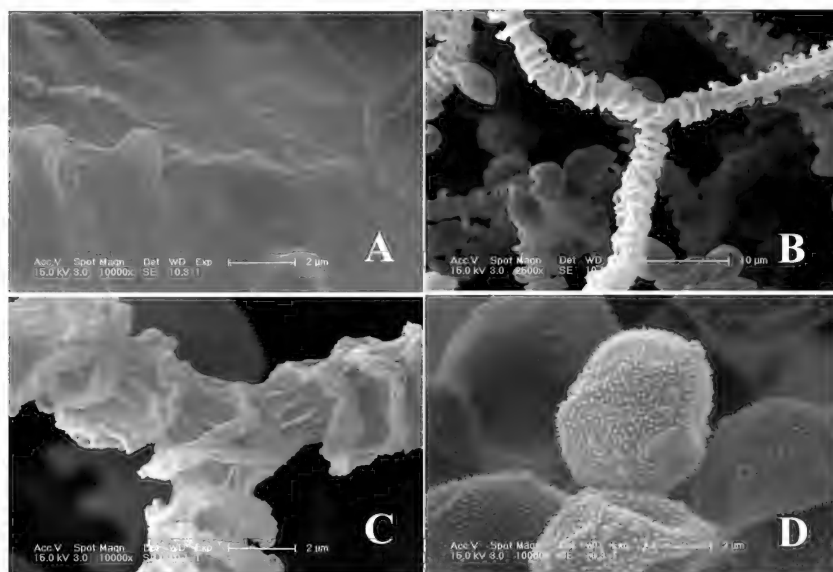


PLATE 2. *Arcyria galericulata* (Isotype): A, part of calyculus, showing many prominent warts and low irregular ridges forming a net with small protuberances. B–C, part of capillitium, showing the ornamentation. D, spores.

papilla is smaller, the sporocarp is larger (about 4.4×1.0 mm after capillitial expansion), the capillitium is marked with spines occasionally interrupted by rings that form characteristic triangular loops, and the spores are smaller (~ 5 μ m diam.) and double-walled.

The capillitial ornamentation of *A. galericulata* resembles that of *A. affinis* Rostaf., *A. helvetica* (Meyl.) H. Neubert et al., and *A. ferruginea* Saut. *Arcyria affinis* is distinguished by its reddish brown sporocarps (about 3–4 mm after capillitial expansion), a capillitium broken away leaving only a few ends attached to the stalk apex, and larger spores (about 7–9 μ m in diam.) (Rostafinsky 1875). *Arcyria ferruginea* differs in its shorter stalk (about 0.2–0.6 mm long), capillitium tube 5–8(–10) μ m in diam., and larger spores (9–12 μ m in diam.) (Sauter 1841), while *A. helvetica* is differentiated by a deeply funnel-shaped calyculus and capillitium only attached to the calyculus at the center (Neubert et al. 1989).

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New records of *Puccinia* species on *Poaceae* from Fairy Meadows, PakistanN.S. AFSHAN^{1*}, A.N. KHALID² & A.R. NIAZI²¹*Centre for Undergraduate Studies & ²Department of Botany,

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ABSTRACT — During a survey of rust fungi of Fairy Meadows, *Puccinia brachypodii* var. *major* on *Poa attenuata* and *P. substriata* var. *indica* on *Pennisetum orientale* were reported for the first time. These new rust fungi records bring to 70 the number of *Puccinia* species reported on *Poaceae* from Pakistan. *Puccinia brachypodii* var. *poae-nemoralis* and *P. poarum* are also reported for the first time from Fairy Meadows.

KEY WORDS — *Anthoxanthum odoratum*, graminicolous rust, Hunza

Introduction

This paper continues our study of graminicolous rust fungi from Pakistan. Previously, about 100 species of graminicolous rust fungi including 68 taxa of *Puccinia* have been reported from Pakistan (Afshan et al. 2010, 2011a,b). During a 2007 survey of the rust flora of Fairy Meadows, four members of *Poaceae* infected with rust fungi were collected. Among these, *Puccinia brachypodii* var. *major* on *Poa attenuata* and *P. substriata* var. *indica* on *Pennisetum orientale* represent new records for Pakistan, while *P. brachypodii* var. *poae-nemoralis* on *Anthoxanthum odoratum* and *P. poarum* on *Poa pratensis* are additions to the rust flora of Fairy Meadows. *Anthoxanthum odoratum* represents a new host for rust fungi from Pakistan.

Materials & methods

Freehand sections of infected tissue and spores were mounted in lactophenol and gently heated to boiling. The preparations were observed under a NIKON YS 100 microscope. Drawings of spores and paraphyses were made using a Camera Lucida (Ernst Leitz Wetzlar, Germany). Spores were measured using an ocular micrometer. At least 25 measurements per fungal structure were taken. Both light microscope and scanning electron microscope (SEM) images were obtained of the spores. Collections

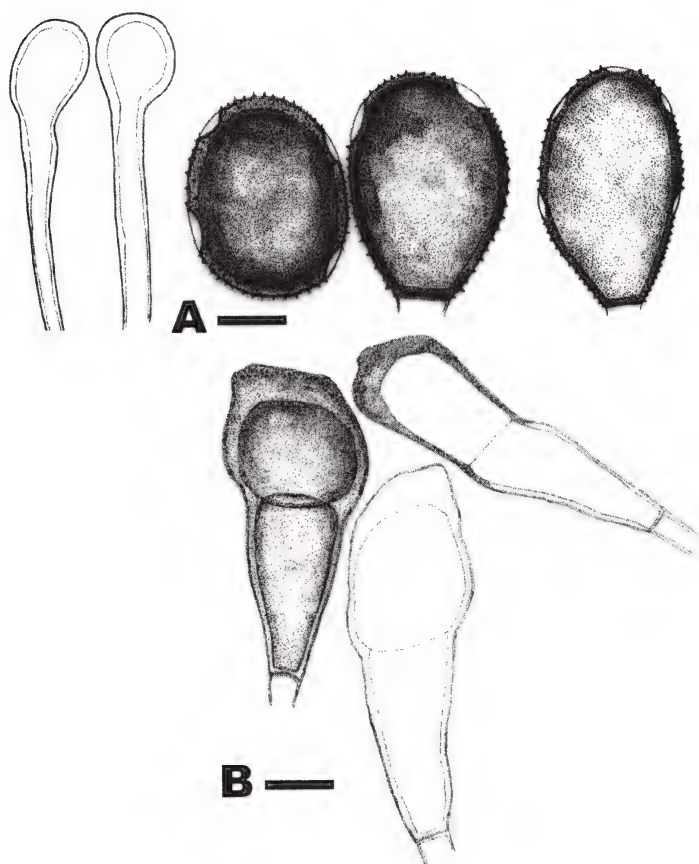
have been deposited in the herbarium of the Botany Department, at the University of the Punjab, Lahore (LAH).

Enumeration of taxa

Puccinia brachypodii var. *major* Cummins & H.C. Greene, Mycologia 58: 711 (1966)

FIGS A-B

SPERMOGONIA and AECIA unknown. UREDINIA amphigenous, subepidermal, light brown, $0.6\text{--}0.8 \times 1.9\text{--}2.0$ mm. UREDINIOSPORES globose to subglobose or obovoid, $(16\text{--})21\text{--}24(\text{--}29) \times (23\text{--})27\text{--}35(\text{--}39)$ μm (mean 22×30 μm); wall $1.5\text{--}2$ μm thick, light yellow to pale brown, echinulate; germ pores (6–)



FIGS. A-B: *Puccinia brachypodii* var. *major*.
A: Urediniospores and paraphyses. B: Teliospores. Scale bars = 10 μm .

8–11, scattered, obscure; pedicel hyaline, 6–8 μm wide and 32–50 μm long. PARAPHYSES 70–95 μm long, hyaline to pale yellow, capitate, head 15–17 μm wide; wall ≥ 2 μm thick. TELIA amphigenous, sub-epidermal, loculate, with stromatic paraphyses, dark brown, $0.4\text{--}0.7 \times 0.6\text{--}0.8$ mm. TELIOSPORES 1–2 celled, oblong to clavate, golden brown to chestnut brown but paler basally, smooth; $20\text{--}26 \times (27\text{--})39\text{--}43(\text{--}55)$ μm (mean 26×34 μm); wall 1.5–2 μm thick; apex truncate or rounded, sometimes conical, 3–5 μm thick; germ pore 1 per cell, obscure; pedicel short, hyaline, $6\text{--}10 \times 31\text{--}45$ μm .

MATERIAL EXAMINED: PAKISTAN, NORTHERN AREAS, Fairy Meadows, at 3036 m a.s.l., stages II + III, on *Poa attenuata* Trin., 12th August, 2007, NSA # 52. (LAH NSA1034).

COMMENTS: *Puccinia brachypodii* var. *major* is a new record for Pakistan, and *Poa attenuata* is a new host record for this fungus. Previously this rust has been reported on *Poa horridula* and *P. candamoana* from Peru (Cummins 1971).

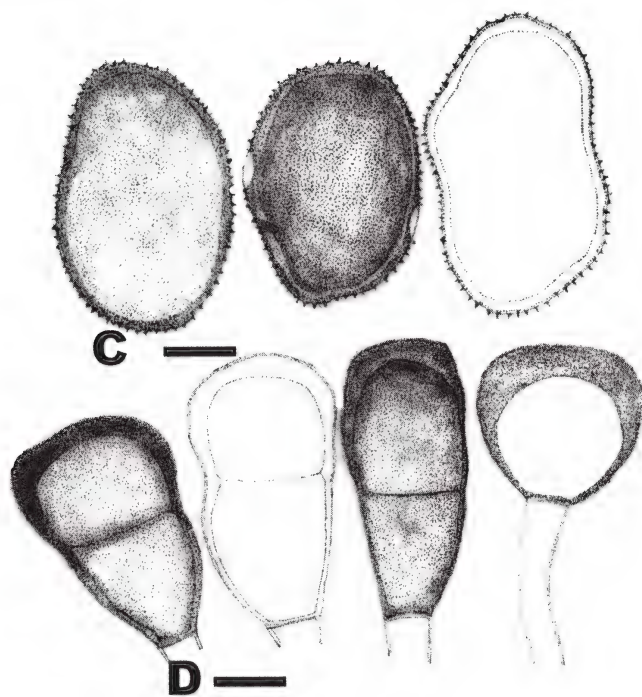
In Pakistan, *Puccinia brachypodii* var. *brachypodii* has been reported on *Brachypodium* sp. from KPK and Kaghan Valley (Kakishima et al. 1993b); and *P. brachypodii* var. *poae-nemoralis* on *Agrostis munroana*, *Poa nemoralis*, *P. pratensis*, and *P. sterilis* from Kaghan Valley, Sharan, Swat, and Azad Jammu & Kashmir (Ahmad 1956a; Kakishima et al. 1993a,b; Masood et al. 1995).

Puccinia substriata var. *indica* Ramachar & Cummins, Mycopath. Mycol. appl. 25: 30 (1965) FIGS C–D

SPERMOGONIA and AECIA unknown. UREDINIA amphigenous, sub-epidermal, yellowish-brown to golden-brown, $0.09\text{--}0.1 \times 0.1\text{--}2.0$ mm. UREDINIOSPORES variable in shape, globose to subglobose or ovoid to ellipsoid, $18\text{--}24(\text{--}29) \times 21\text{--}24(\text{--}30)$ μm , pale brown to golden brown, echinulate; wall 1–1.5 μm thick; germ pores 3–4(–5), equatorials; pedicel hyaline, 4–5 μm wide and ≥ 15 μm long. TELIA amphigenous, mostly adaxial, sub-epidermal, erumpent, dark brown to blackish brown, $0.09\text{--}0.5 \times 0.2\text{--}0.6$ mm. TELIOSPORES 1–2-celled, oblong to clavate, $17\text{--}24(\text{--}29) \times (29\text{--})34\text{--}52(\text{--}60)$ μm , cinnamon brown to golden brown, paler basally, smooth; wall 2–3 μm thick; apex mostly truncate, sometimes rounded, 4–9 μm thick; germ pores obscure; pedicel hyaline to light brown, $6\text{--}8 \times 8\text{--}12$ μm .

MATERIAL EXAMINED: PAKISTAN, NORTHERN AREAS, Fairy Meadows, at 3036 m a.s.l., stages II + III, on *Pennisetum orientale* Rich., 12th August, 2007, NSA # 70. (LAH NSA1095).

COMMENTS: *Puccinia substriata* var. *indica* is a new record for Pakistan. The variety has been reported from India on *Pennisetum typhoides* (Cummins 1971), and Afshan et al. (2008) reported another variety, *P. substriata* var. *insolita* (on *Panicum antidotale*) from Munchinabad in Pakistan. *Puccinia penniseti-lanati* (on *P. lanatum*) and *Uromyces penniseti* (on *P. americanum* from Kaghan Valley, Tandojam, and Karachi are two other poaceous rusts known to occur

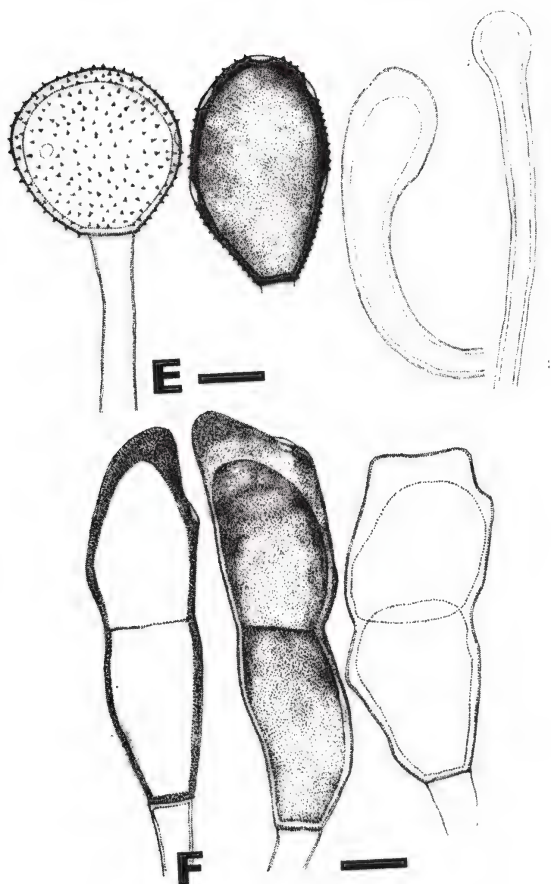


FIGS. C–D: *Puccinia substriata* var. *indica*.
C: Urediniospores. D: 1–2-celled teliospores. Scale bars = 10 μ m.

on *Pennisetum* species in Pakistan (Ahmad 1969; Hasnain et al. 1959; Khan & Kamal 1968).

Puccinia brachypodii var. *poae-nemoralis* (G.H. Otth) Cummins & H.C. Greene,
Mycologia 58: 705 (1966) FIGS E–F

SPERMOGONIA and AECIA unknown. UREDINIA on leaves, mostly adaxial, yellowish or yellowish brown, 0.09–0.2 $\times$ 0.2–0.4 mm. UREDINIOSPORES obovoid or ellipsoid to broadly ellipsoid, 19–26 $\times$ 23–36 μ m (23.2 $\times$ 28.6 μ m); wall 2–3 μ m thick, light brown to cinnamon brown, closely echinulate; germ pores 5–9, obscure; pedicel hyaline, short, 7–8 $\times$ 38–45 μ m. PARAPHYSES cylindric to capitate, hyaline or yellowish, mostly 80–100 μ m long and 14–18 μ m wide, usually geniculata, wall 2–4 μ m thick throughout or to 6 μ m thick in the head. TELIA on leaves, mostly abaxial, sub-epidermal, 0.06–0.1 $\times$ 0.1–0.2 mm, blackish, loculate with a few brown paraphyses surrounding the sori. TELIOSPORES 1–2 celled (few one-celled spores also observed), oblong to clavate, 13–24 $\times$ 40–59(–65) μ m (mean 17.7 $\times$ 50.6 μ m), not or slightly constricted at



FIGS. E–F: *Puccinia brachypodii* var. *poae-nemoralis*.
E: Urediniospores and paraphyses. F: Teliospores. Scale bars = 10 μ m.

the septum; wall 1–1.5 μ m thick, smooth, brown to chestnut brown or paler basally; apex 5–9 μ m thick, truncate or conical; germ pore 1 per cell, upper sub-apical, lower at the equator or near the septum; pedicel short, light brown, 5–6 $\times$ 9–15 μ m, not collapsing, thick walled.

MATERIAL EXAMINED: PAKISTAN, NORTHERN AREAS, Karimabad, Hunza, at 2438 m a.s.l., stages II + III, on *Anthoxanthum odoratum* L., 13th August, 2007; Fairy Meadows, at 3036 m a.s.l., 12th August, 2007, NSA # Gr 58 (G06). (LAH NSA1035a & 1035b).

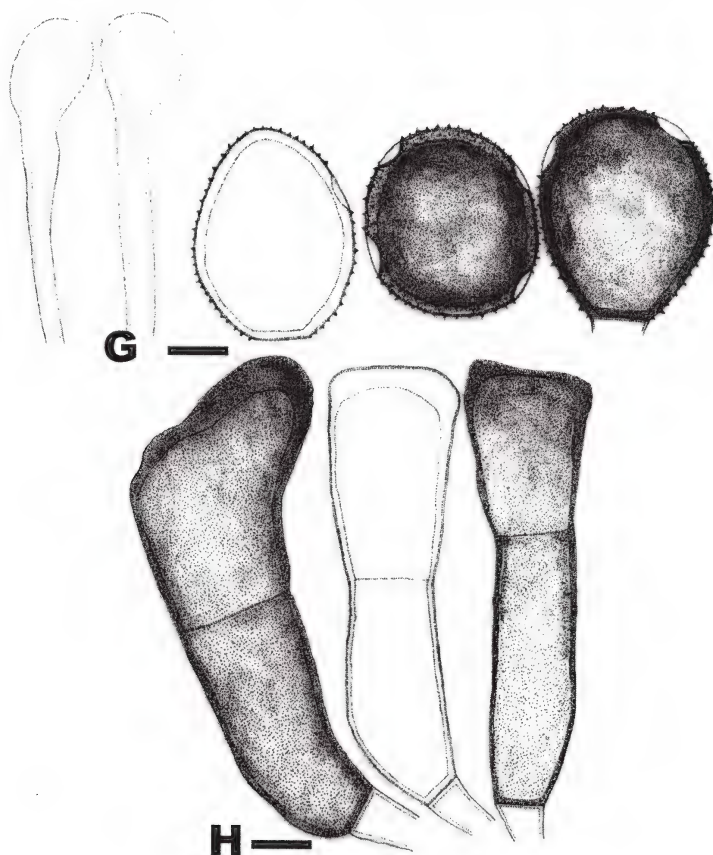
COMMENTS: *Puccinia brachypodii* var. *poae-nemoralis* has been reported on leaves of *Agrostis munroana*, *Poa nemoralis*, *P. pratensis*, and *P. sterilis* from Kaghan Valley, Sharhan, Swat and Azad Jammu & Kashmir (Ahmad et al. 1997).

P. brachypodii var. *poae-nemoralis* is reported for the first time from Fairy Meadows and Hunza, Northern Areas of Pakistan; *Anthoxanthum odoratum* is a new host for rust fungi from Pakistan.

Puccinia poarum P. Nielsen, Bot. Tidsskr., ser. 3, 2: 34 (1877)

FIGS G–H

SPERMOGONIA and AECIA unknown. UREDINIA amphigenous, subepidermal, light yellow to light brown, 0.6–0.8 × 1.9–2.0 mm. UREDINIOSPORES globose to subglobose or obovoid, 20–26 × 23–30 µm (mean 22.92 × 26.71 µm); wall 1.5–2 µm thick, light yellow to pale brown, echinulate; germ pores 4–5(–8), obscure; pedicel hyaline, 5–6 µm wide and ≥ 15 µm long. PARAPHYSES few, clavate to capitate, peripheral, hyaline to pale yellow, head 19–21 µm wide, wall ≥ 2 µm thick, 94–100 µm long. TELIA amphigenous, covered by the epidermis, rarely



FIGS. G–H: *Puccinia poarum*.

G. Urediniospores and paraphyses. H: Teliospores. Scale bars = 10 µm.

loculate, dark brown, $0.4\text{--}0.7 \times 0.6\text{--}0.8$ mm. TELIOSPORES 1–2 celled, oblong to clavate; wall $2\text{--}3$ μm thick, golden brown to chestnut brown but paler basally, smooth; $11\text{--}19(\text{--}22) \times 37\text{--}58(\text{--}67)$ μm (mean 15.7×47.9 μm); apex truncate or conical to obliquely conical, $3\text{--}5$ μm thick; germ pore 1 per cell, obscure; pedicel short, brown, $6\text{--}7 \times 9\text{--}11$ μm .

MATERIAL EXAMINED: PAKISTAN, NORTHERN AREAS, Fairy Meadows, at 3,036 m a.s.l., stages II + III, on *Poa pratensis* L., 12th August, 2007, NSA # G 371 (LAH NSA1077).

COMMENTS: *Puccinia poarum* has been reported on *Poa annua* from Lahore by Ahmad (1956a,b), on *Agrostis* sp. from Kaghan valley and Azad Jammu & Kashmir by Kakishima et al. (1993b). *Puccinia poarum* is newly recorded from Fairy Meadows, Northern areas of Pakistan.

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Lectotypification and characterization of the natural phenotype of *Fusarium bactridioides*¹

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ABSTRACT — Specimens of *Fusarium bactridioides* deposited in the US National Fungus Collections and New York Botanical Garden were examined and a lectotype is selected based on naturally infected galls of *Cronartium conigenum* collected in Arizona. This species was previously only described from culture, and its natural phenotype is presented and illustrated here. Historical experiments involving the attempted use of *F. bactridioides* as a biocontrol of pine blister rusts in Oregon and New Hampshire are reviewed, but the unpublished records of the ultimate fate of these experiments could not be located.

KEY WORDS — *Hypocreales*, *Nectriaceae*, anamorph taxonomy, biological control

Introduction

Fusarium bactridioides was the last species of the genus to be formally described in the august pages of the journal *SCIENCE*. The single page outlined the circumstances of the discovery of the fungus by the American botanist Arthur Hinckley and forest pathologist L.N. Goodding. The *Fusarium* “thoroughly parasitized” a cone of *Pinus leiophylla* var. *chihuahuana* (Chihuahua pine) attacked by the cone blister rust, *Cronartium conigenum* Hedgc. & N.R. Hunt. It was originally collected in the remote Chiricahua Mountains in Arizona (ca. 31°50'N 109°17'W), a so-called ‘sky island’ range of eroded volcanic rhyolite rock arising from the surrounding grassland desert.

¹ This article, the first of a projected series on the typification and nomenclature of species of *Fusarium* and allied genera, is dedicated to our friend and mentor Emory G. Simmons, whose erudite and articulate ‘*Alternaria*: Themes and Variations,’ published in this journal, inspired this pale imitation.

Following the formal description is a remarkable story of what must be among the earliest deliberate releases of one fungus as a possible biological control of another. The *Cronartium* host is one of several rusts infecting *Pinus* species in the southwestern United States and adjacent Mexico, with uredinia and telia developing on various *Quercus* spp. (Cannon 2007). In July 1932, only three months after the discovery of the species, conidia of *F. bactridioides* were sprayed onto living *C. conigenum* cankers on *Pinus monticola* in Clackamas County Oregon. The following July, all the inoculated cankers were dead. Inoculations were then attempted on galls of *C. harknessii* E. Meinecke and *C. filamentosum* Hedgc. on *P. contorta* in two locations in Oregon, where sporulating *Fusarium* colonies were observed on the galls during a survey four months later. Field inoculations in four localities in Idaho were reported, but only sketchy details were provided of observations of the *Fusarium* sporulating on the cankers afterwards and there are no subsequent literature reports. Unfortunately, we were unable to locate any unpublished notes of L.N. Goodding that might give more information on the ultimate fate of these inoculations.

Fusarium bactridioides was classified in *Fusarium* sect. *Discolor* by Wollenweber (1934), Wollenweber & Reinking (1935), and Gerlach & Nirenberg (1982). It was considered a synonym of *F. sambucinum* Fuckel (Booth 1971, Nelson et al. 1983) and *F. bactridioides* vaguely fits the broad concept of *F. sambucinum* adopted by these authors. However, the macroconidia lack the asymmetrical, 'dolphin-nose' apical cell typical of the latter species. Of the species segregated from *F. sambucinum* by Nirenberg (1985), the macroconidia of *F. bactridioides* have some morphological similarity to those of the potato pathogen *F. venenatum* Nirenberg, but the latter species lacks microconidia. The phylogenetic and taxonomic studies of O'Donnell et al. (1998) and Nirenberg & O'Donnell (1998) accept *F. bactridioides* as a distinct species in the American clade of the *Fusarium fujikuroi* complex.

Wollenweber (1934) did not illustrate the species with the protologue, but later published a drawing as *Fusarium autographice delineata* no. 1153 (Wollenweber 1935), which was excerpted in his subsequent monographs. All published descriptions and illustrations of this species are probably based on one culture, exemplified by the modern species description and microscopic photographs of Gerlach & Nirenberg (1982: 267). This *in vitro* characterization is not repeated here, but the natural 'wild type' of this species on pine rust galls is illustrated and described. No holotype was designated in the protologue, and this shortcoming is remedied below by the selection of a lectotype.

Typification

The protologue of *F. bactridioides* explicitly mentions three specimens, the first probably the original collection from Arizona, and two from the inoculation



FIGS. 1–2. *Fusarium bactridioides*, habit photographs. 1. Naturally infected *Cronartium conigenum* gall (BPI 451322). 2. Detail of sporodochia (lectotype, composite photograph assembled with CombineZ, Hadley 2006).

experiments in Oregon. There is no designation of a holotype. The description was based on a culture, and it is unlikely that Wollenweber ever saw any of the specimens that he listed.

Eight specimens of *F. bactridioides* are deposited in BPI and one in NY. Apart from one specimen collected and identified by J.R. Hansbrough in Waterville, New Hampshire (BPI US0451805) after the publication of the protologue, these collections are authentic and represent; a) material collected by A. Hinckley in the Chiricahua Mountains (NY 00936830 from 1932 on a host identified as *Cronartium quercuum* (Berk.) Miyabe; BPI US0451322 from 1933 on a host identified as *C. conigenum*); b) dried cultures from “cones collected by Arthur, Mar. 15, 1934” (BPI US0451803) and four specimens collected in 1933 from the inoculated locations in Oregon. It seems reasonable to designate the galls of *C. quercuum* collected by Hinckley in 1932 as the lectotype, despite the absence of the variety name for the host. The dates and locality match precisely and the identification of the host probably was changed between the times of specimen deposit and publication. This is presumably the specimen from which the cultures and subsequent specimens from inoculated localities were derived. The 1933 specimen in BPI appears also to be a natural infection from the type locality and although it predates publication, it is not mentioned in the protologue.

An argument could be made to designate the dried cultures in US0451803 as the lectotype, because they probably represent the strain(s), but not the actual transfers, that Wollenweber described. Unfortunately, the six dried PDA slants have no evident sporodochia or macroconidia, although there are abundant

microconidia in the cottony, white aerial mycelium. The cultures are heavily contaminated with coccoid bacteria. The date on the package containing the dried cultures is "Mar. 15, 1934", a scant three months before Wollenweber's description was published. The notes inside this packet suggest that the 1934 date is actually when the preserved cultures were dried. Only one strain of this fungus now exists, i.e. Wollenweber 4748 → CBS 177.35 (→ BBA 63602, CBS → NRRL 22201, NRRL → DAOM 225115, NRRL → CBS 100057). The data for CBS 100057 record *P. leiophylla* as the plant host and the location as Arizona, suggesting that this strain, which was regarded as an 'ex-type culture' by Gerlach & Nirenberg (1982) and Nirenberg & O'Donnell (1998), was derived from material from the Chiricahua Mountains. The protologue is ambiguous about how many strains were originally isolated and how many were sent to Wollenweber by his American colleagues. One of these strains was illustrated as *Fusarium autographice delineata* no. 1153, and we must assume that the culture now represented by CBS 100057 is the same strain. We consider this to be the ex-lectotype strain, and see no need to formalize this by epitypification.

For formal typification, we prefer to emphasize the concept of the species as a parasite of *Cronartium*, and designate the lectotype accordingly below.

Taxonomy

Fusarium bactridioides Wollenw., Science 79: 572. 1934.

FIGS 1–5

MYCOBANK MB 258078

TYPE: On *Cronartium quercuum* on *Pinus chichuahua* (sic), USA, Arizona, Chiricahua Mt., Cave Creek, Ill. 1932, Arthur Hinckley (lectotype designated here, NY 00936830).

Sporodochia are erumpent from host tissue and visible as Orange-White (5A2, Kornerup & Wanscher 1978) to Light Orange (5A5) masses of conidial slime. On the naturally infected specimens, they are irregular in outline and form lesions up to 1 cm × 5 mm on the galls. On inoculated specimens, the lesions are scattered and smaller, about 200–1000 µm diam. The stroma is poorly developed, about 100 µm thick, and in optical section appears as a textura angularis of thin- to slightly thick-walled cells about 3.5–7 µm wide, but its hyphal character is evident with changes of focus. A hymenium-like layer of conidiophores and phialides arises from the stroma, with the conidiophores more or less biverticillate, but with phialides also often arising at the first level of branching. Metulae are cylindrical, doliiform to slightly clavate, 8.5–11.5 × 3.5–7 µm at the broadest part. Phialides are 9.5–15 × 3–5 µm at the broadest part, narrowly doliiform, sometimes with a central constriction, in terminal pairs or whorls of 3, or arising singly, in pairs or in whorls with metulae, with a flared collarette about 1–1.5 µm long and periclinal thickening sometimes visible; conidiogenous aperture about 2 µm wide.



FIGS 3–4. Microphotographs of *Fusarium bactridioides*, BPI 451804. 3. Macroconidia. (composite image). 4. Hand section of the sporodochial stroma, showing conidiophores and phialides. Scale bars = 10 μ m.

There is no obvious distinct difference between ‘microconidial’ and ‘macroconidial’ sporodochia and there is more or less a continuum between the two conidial types. Few macroconidia occur on the naturally infected

specimens, but they are more abundant on the lectotype and inoculated specimens. Macroconidia are 3–6 septate; 3-septate predominate and are $30.5\text{--}45.5 \times 4.5\text{--}6 \mu\text{m}$ (mean $\pm$ SE = $38.3 \pm 0.8 \times 5.3 \pm 0.1$, $n = 20$), $l/w = 5.5\text{--}9$; 4-septate $27\text{--}36.5 \times 5\text{--}6 \mu\text{m}$ (mean $\pm$ SE = $31.9 \pm 0.8 \times 5.4 \pm 0.1$, $n = 10$), $l/w = 5\text{--}7$; 5-septate $29\text{--}39.5 \times 5.5\text{--}6.5 \mu\text{m}$ (mean $\pm$ SE = $34.6 \pm 0.9 \times 5.8 \pm 0.1$, $n = 13$), $l/w = 5\text{--}7$; 6-septate $35.5\text{--}41 \times 5\text{--}6 \mu\text{m}$ ($n = 3$), $l/w = 6\text{--}7$. In side view, the ventral surface is more or less straight or gently curved, and the dorsal surface is moderately curved, with the walls more or less parallel in the central two cells of the conidia, with the widest point near the middle or above the middle. The apical cell is bluntly rounded, and roughly the same length as the penultimate cell. The basal cell is tapered more acutely than apical cell; the base is rounded, flat, or has a slight indentation on the dorsal side or central papilla, indicating a foot cell. In front view, the macroconidia appear somewhat clavate. 4–6 septate macroconidia tend to have unequal lengths of cells, with the additional septa dividing one but not all of the original four cells. Microconidia are abundant in the sporodochia, are 0–3 septate, and vary in shape and size from small, ellipsoidal, oblong-ellipsoidal or allantoid cells that are obviously microconidia to fusiform to clavate, septate spores that intergrade with macroconidia. Aseptate conidia $6\text{--}18 \times 3.5\text{--}6.5 \mu\text{m}$ (mean $\pm$ SE = $11.8 \pm 0.6 \times 4.9 \pm 0.1$, $n = 25$), $l/w = 1.5\text{--}4$. 1-septate conidia $9.5\text{--}19.5 \times 4\text{--}6.5 \mu\text{m}$ (mean $\pm$ SE = $14.9 \pm 0.5 \times 5.1 \pm 0.1$, $n = 25$), $l/w = 2\text{--}4$. 2-septate conidia are infrequent and $15\text{--}24 \times 5\text{--}6.5 \mu\text{m}$. 3-septate microconidia also occur and can be distinguished from macroconidia by their shorter length and their more clavate shape; they are $16\text{--}31 \times 5\text{--}7 \mu\text{m}$ (mean $\pm$ SE = $24.0 \pm 0.7 \times 6.1 \pm 0.1$, $n = 25$), $l/w = 3\text{--}5$. In general, the bases of microconidia are conical, symmetrical or asymmetrical, with a flat secession scar or relatively conspicuous papilla; the apical cell or part of the conidium is rounded.

ADDITIONAL SPECIMENS EXAMINED: USA, Arizona, Chiricahua Mt. (as Chiricalma), Cave Creek, on *Cronartium conigenum* on *Pinus leiophylla*, 25.VIII.1933, Arthur Hinckley (BPI US0451322); Oregon, Mt. Hood National Forest, On *Cronartium harknessii* on *Pinus contorta*, 8.VII.1933, L.N. Goodding (BPI US0451323); on *Cronartium ribicola*, 24.X.1933, L.N. Goodding (BPI US0451325); Eagle Creek, on *Cronartium ribicola* on *Pinus monticola*, 24.X.1933, L.N. Goodding (BPI US0451326); six dried slant cultures on PDA from “cones collected by Arthur” (BPI US0451803); Hood River Co., Eagle Creek, on *Cronartium ribicola* on *Pinus* sp., 24.X.1933, L.N. Goodding (BPI US0451804); New Hampshire, Waterville, on *Pinus strobus*, 1.IX.1935, J.R. Hansbrough (BPI US0451805).

Discussion

The micromorphology of *F. bactridioides* reported here from natural material is comparable to that reported from cultures of this species by Wollenweber (1934) and Gerlach & Nirenberg (1982). The main distinction is that on the natural substrate the microconidia are produced from the

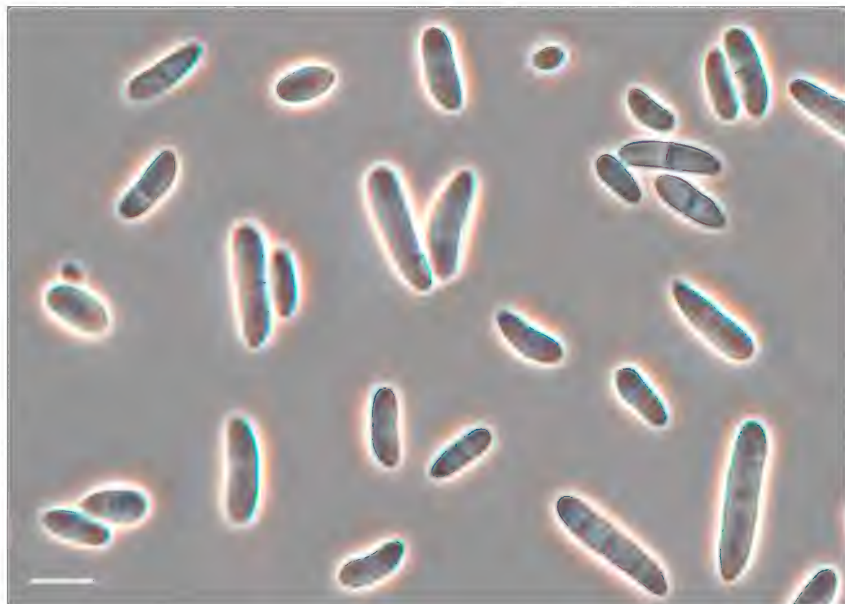


FIG. 5. Microphotograph of microconidia of *Fusarium bactridioides*, lectotype, NY 00936830 (composite image). Scale bar = 10 μ m.

same sporodochia as the macroconidia, whereas in culture microconidia are produced independently. In nature, there is a continuum of shape and size between macro- and microconidia, but in culture the two kinds seem to be morphologically distinct. Gerlach & Nirenberg (1982) reported macroconidia with up to 11 septa in culture, with 3–5 septa being the typical condition. On the specimens, the macroconidia with three septa predominated, with a small number of 4–6 septate macroconidia seen on some specimens. Morphologically, *Fusarium bactridioides* has more robust macroconidia than is typical for the *F. fujikuroi* complex, and the 0–1 septate oval macroconidia are produced on the agar surface *in vitro*, rather than in the aerial mycelium as they are in related species. The mycoparasitic habit is also unusual in this group.

To our knowledge, *Fusarium bactridioides* has not been collected after 1935 and no natural infections have been reported outside of Arizona. The Hansbrough specimens (USO451805) collected in 1935 are the result of inoculation experiments in New Hampshire in 1934, about which apparently nothing was ever published. We were unsuccessful in locating the typewritten report by Hansbrough cited in a note included with the specimens. Whether *F. bactridioides* is indigenous and perhaps restricted to the remote Chiricahua Mountains, and whether the inoculations of blister rust cankers in Oregon,

Idaho and New Hampshire still persist, seem questions worthy of investigation. Such inoculation experiments would not easily be conducted today, with the need for risk assessments and legal permits from regulatory agencies. *Fusarium bactridioides* may provide a unique opportunity to search for the lingering fingerprints of innovative scientific activities of a more innocent time.

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We are grateful to Helgard Nirenberg and Walter Gams for their insights into the practices of H.W. Wollenweber in Berlin, the curators of BPI and NY for the kind loans of specimens discussed in this article, and Drs T. Aoki and B. Summerell for pre-submission reviews. We appreciate the efforts of staff at the herbarium (S. Hunkins) and library (M. Carpenter) of the University of Arizona to find archival material of L.N. Goodding, and staff at the Connecticut Agricultural Experiment Station (D.W. Li, S. Anagnostakis, V. Bomba-Lewandoski, W. Elmer) and USDA Forestry Lab in Hamden, CT (M. Keena) for searching for archives of J.R. Hansbrough.

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Lectotypification of *Crepidotus variabilis* var. *subsphaerosporus*SOŇA JANČOVIČOVÁ¹ & SHAUN R. PENNYCOOK²¹ Comenius University in Bratislava, Faculty of Natural Sciences,
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ABSTRACT — An original watercolour painting by J.E. Lange is designated as the lectotype of *Crepidotus variabilis* var. *subsphaerosporus*. This painting was cited in two descriptions published invalidly by Lange prior to his validation of the name; it therefore constitutes part of the original material on which he based his description. As a lectotype, it supersedes the neotype previously designated by Senn-Irlet, which is here designated as epitype.

KEY WORDS — Afbildninger af Danmarks Agaricaceer, Basidiomycota, *Crepidotus cesatii* var. *subsphaerosporus*, *Crepidotus kubickae*, Flora Agaricina Danica

Introduction

The name *Crepidotus variabilis* var. *subsphaerosporus* was first proposed by J.E. Lange (1938: 52, as ‘*subsphaerospora*’) as a nomen non rite publicatum, lacking a Latin description (McNeill et al. 2006: Art. 36.1). No type specimen was designated; according to M. Lange (1969: 131), J.E. Lange “never tried to make up a set of herbarium specimens.” However, an illustration was cited (as “D.A. pl. 533”), together with details of collection locality, habitat, and date. The abbreviation “D.A.” refers to the extensive series of watercolours painted by J.E. Lange during the years 1893–1910 and compiled under the title “Afbildninger af Danmarks Agaricaceer” (J.E. Lange 1914: 1; M. Lange 1969: 122–123). The original paintings (initially held by J.E. Lange) are now conserved in the Herbarium, Natural History Museum of Denmark, University of Copenhagen (C), and are reproduced on the Flora Agaricina Danica website (<http://130.225.211.158/agaricina/flagar-search.htm>; H. Knudsen, pers. comm.). Subsequently, J.E. Lange painted a duplicate set for the Museum on better quality paper (M. Lange 1969: 123); this set is now held in the private collection of his granddaughter, Dr. Lene Lange, Aalborg University (H. Knudsen, pers. comm.).

J.E. Lange (1939: 46, pl. 133 fig. E) republished the description with minor changes accompanied by a printed reproduction of the original painting (with some repositioning of component elements), but the name remained invalid for lack of a Latin description.

J.E. Lange (1940: IV) finally validated the name in a set of Latin diagnoses of names invalidly published in “Flora Agaricina Danica.” Although there is no direct reference to the earlier publication pages, the trinomial is marked with an asterisk, indicating that the name had originally been published in “Studies in the Agarics of Denmark” during the period “1935–40” (J.E. Lange 1940: II), i.e., in the series of papers that included J.E. Lange (1938).

When Senn-Irlet (1995: 53) recombined the name as *Crepidotus cesatii* var. *subsphaerosporus* (J.E. Lange) Senn-Irlet, she designated a Swiss collection as neotype. However, this designation ignored the availability of original material (the watercolour painting) that should have been selected as lectotype (McNeill et al. 2006: Art. 9.10, 9.11).

Here we designate the original watercolour painting as lectotype, and the superseded Swiss “neotype” as epitype.

Taxonomy and typification

The name *Crepidotus variabilis* var. *subsphaerosporus* has priority at variety rank (e.g., as treated in Senn-Irlet 1995). Ripková (2009: 272) summarized the nomenclatural history of the taxon under *Crepidotus kubickae*, the synonym that has priority at species rank. Two widely cited purported recombinations of J.E. Lange’s basionym at species rank were not validly published (McNeill et al. 2006: Art. 33.4): “*Crepidotus subsphaerosporus*” (J.E. Lange) Kühner & Romagn. (Kühner & Romagnesi 1953: 76) lacked a full and direct basionym reference, and “*Crepidotus subsphaerosporus*” (J.E. Lange) Kühner & Romagn. ex Hesler & A.H. Sm. (Hesler & Smith 1965: 121) gave a full and direct reference to the 1938 nomen non rite publicatum, a non-correctable nomenclatural error (McNeill et al. 2006: Art. 33.7).

J.E. Lange preserved no herbarium specimens other than an occasional spore print. There are no J.E. Lange collections or spore prints of *C. variabilis* var. *subsphaerosporus* at C; the watercolour painting, conserved at C (viewable on <http://130.225.211.158/agaricina/flagar-search.htm> under “*Crepidotus subsphaerosporus*”), is apparently the only extant original material. (J.E. Lange’s duplicate painting is extant, but it is debatable whether this can be considered to be “original material.”) The original painting is on a 26.5 × 17.7 cm sheet of thick, cardboard-like, aquarelle paper, which has now yellowed considerably. The images comprise a drawing of two basidiospores, a painted representation of the spore print colour (easily overlooked because of its resemblance to adjacent blotches of foxing discoloration), and three habit paintings of the basidiomata.

(The three habit paintings and the basidiospore drawing, but not the spore print colour representation, are also reproduced in J.E. Lange 1939: pl. 133 fig. E.) The original painting bears various handwritten ink and pencil annotations in Danish: in the upper right corner “Tavle 533”; below the basidiospore drawing “sp x 1000”; and along the lower margin “*Crepidotus sessilis* (Bull.) Schroet. f. *subsphaerospora*. Sporestøv blegt okkerbrunt. Sp sphaerisk-ellips, 6 x 4 $\frac{3}{4}$. Hæsbjerg, på pinde af eg o.a. løvtræer. Okt. 1901.” Hæsbjerg is an old spelling for the modern Hesbjerg, the name of a number of localities on Funen, the Danish island where J.E. Lange lived and did most of his collecting; the most likely collection locality is at 55°22'N 10°13'E, the only forested Hesbjerg on Funen (H. Knudsen, pers. comm.).

We propose the original painting as lectotype of *C. variabilis* var. *subsphaerosporus*. We also propose Senn-Irlet's superseded “neotype” as epitype, providing micromorphological data to supplement the macromorphology illustrated in the lectotype.

Crepidotus kubickae Pilát, Stud. Bot. Čechoslov. 10: 150, 1949, as ‘*Kubičkae*'.

TYPE (Pilát 1949, Senn-Irlet 1992, Ripková 2009): CZECH REPUBLIC. Central Bohemia: “Poříčko nad Sázavou, ad terram inter muscis, 29.V.1949, legit J. Kubička, det. Pilát, Typus.” (PRM 665190, **holotype**).

= *Crepidotus variabilis* var. *subsphaerosporus* J.E. Lange, Fl. Agaric Danic. 5: IV, 1940.

TYPES: DENMARK. Funen: Hesbjerg, “*Crepidotus sessilis* (Bull.) Schroet. f. *subsphaerospora*. Sporeprint pale ochre brown. Sp sphaerical-ellipsoid, 6 x 4 $\frac{3}{4}$. Hæsbjerg, on sticks of *Quercus* and other deciduous trees. Oct. 1901.” [in Danish]. (C, Danmarks Agaricaceer Tavle 533, original watercolour painting by J.E. Lange, **lectotype designated here**). SWITZERLAND. Bern: Röthenbach, Schineggswand am Schallberg, alt. 1000 m, *Abieti-Fagetum*, on fallen *Picea* twigs, 11 Oct. 1989, Senn-Irlet 89/240 (G, **epitype designated here**).

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MYCOTAXON

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A new species of *Conidiobolus* (Ancylistaceae) from Anhui, ChinaYONG NIE¹, CUI-ZHU YU¹, XIAO-YONG LIU^{2*} & BO HUANG^{1*}¹Anhui Provincial Key Laboratory of Microbial Control, Anhui Agricultural University,
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ABSTRACT —*Conidiobolus sinensis* was isolated from plant detritus in Huoshan, Anhui Province, eastern China. It produces primary conidiophores from cushion mycelium, which is distinct from all other species in the genus except *C. stromoideus* and *C. lichenicola*. Morphologically *C. sinensis* differs from *C. stromoideus* in the shape of the mycelia at the colony edge and conidiophore length and from *C. lichenicola* by colony color and mycelial form. A phylogram based on partial 28S rDNA and EF-1 α sequences from 14 *Conidiobolus* species shows *C. sinensis* most closely related to *C. stromoideus*, forming a clade of sister taxa with a 100% bootstrap. DNA similarity levels between these two species were 94% (28S rDNA) and 96% (EF-1 α). Based on the morphological and molecular evidence, *C. sinensis* is considered a new species.

KEY WORDS —*Entomophthorales*, hyphal knots, taxonomy

Introduction

Species belonging to *Conidiobolus* can be easily isolated from soil, decaying leaf litter, rotten vegetables and some dead insects, although the type of the genus, *C. utriculosus* Bref., was first isolated from the decaying fleshy fruitbodies of *Exidia* and *Hirneola*. The genus is diagnosed by (i) nuclei that do not stain in aceto-orcein and lacking obviously granular contents, (ii) simple conidiophores, (iii) globose to pyriform multinucleate conidia, (iv) resting spores formed in the axis of hypha (mostly as zygospores), and (v) walled vegetative cells (Humber 1997). After Huang et al. (2007) recognized 30 species within *Conidiobolus*, only two additional species — *C. margaritatus* (Huang et al. 2007), *C. thermophilus* (Waingankar et al. 2008) — have been added.

While many phylogenetic studies of entomogenous fungi have been conducted in recent years, there is little information regarding the phylogeny

and molecular taxonomy of *Conidiobolus*. The limited SSU analysis of Jensen et al. (1998) suggested that *Conidiobolus* may be polyphyletic. Vilela et al. (2010) were the first to detail the taxonomic and phylogenetic features of three pathogenic *Conidiobolus*: *C. coronatus* (Costantin) A. Batko, *C. lamprauges* Drechsler, and *C. incongruus* Drechsler.

Anhui Province is located at 29°04'–34°06'N 114°09'–119°06'E, eastern China, and most parts of the province have subtropical vegetation, but some temperate vegetation occurs in mountains. Recently, several new fungal species on decaying wood have been reported from the area (Dai 2010; Cui et al. 2011). In the course of studies on *Conidiobolus* species from China, one strain with cushion mycelia was isolated from decaying plant material in Anhui and is described in this report as a new species, based on both morphological and molecular data.

Materials & methods

Morphological studies

Plant detritus was sampled on 17 June 2010 near a reservoir in Huoshan, Anhui, China. The sample was screened for saprotrophic *Conidiobolus* by canopying moistened detritus on agar plates following Drechsler (1952) and King (1976a) to exploit the forcible discharge of *Conidiobolus* conidia. These isolation plates were incubated at 21°C and examined daily for one week. Once *Conidiobolus* cultures were detected on the PDA canopy, they were transferred to new PDA plates for purification and morphological study. The measurements of different fungal structures followed King (1976a).

Molecular studies

The *Conidiobolus* strains used in the molecular study are shown in TABLE 1. Ten ex-type strains of *Conidiobolus* spp. were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), and the remaining *Conidiobolus* strains were obtained from the Research Center for Entomogenous Fungi (RCEF; Anhui Agricultural University, Hefei, China). Genomic DNA was extracted using the CTAB method (Yi et al. 2003). The extracted DNA was stored in 50–100 µL of HPLC-H₂O at -20°C, and was diluted 10-fold with HPLC-H₂O for use in PCR reactions. Regions of two genes were amplified by PCR: 1) nuclear ribosomal large subunit (LSU rDNA) by primers LROR and LR5 (Vilgalys & Hester 1990) and 2) elongation factor 1- α (EF-1 α) by primers EF983 and EF1aZ-1R (<http://www.aftol.org/primers.php>). All procedures used in this study for LSU amplification have been described previously (Liu et al. 2005). The PCR reaction mixture for amplifying EF-1 α contained 200 µM each dNTP, 1× Mg-free buffer, 2.5 mM MgCl₂, 0.5 µM each primers, 1 ng/µL genomic DNA, and 0.04 Unit/L Taq polymerase. The cycle program included initial denaturation at 100°C for 5 min followed by 95°C for 5 min (during which time Taq polymerase was added to each tube), 34 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 2 min, and a final extension at 72°C for 10 min. The nucleotide sequences of the PCR products were determined on both strands by using dideoxy-nucleotide chain termination on an ABI 3700 automated sequencer at Shanghai Genecore Biotechnologies Company. Sequence data of the 19

TABLE 1. *Conidiobolus* and *Entomophthora* cultures and sequences used in phylogenetic analyses.*

FUNGAL TAXON	STRAIN #	28S rDNA	EF-1 α
<i>C. chlamydosporus</i> Drechsler	ATCC12242 (T)	JF816212	JF816234
<i>C. denaerosporus</i> Drechsler	ATCC12940 (T)	JF816215	JF816228
<i>C. firmipilleus</i> Drechsler	RCEF4429	JF816222	JF816237
<i>C. gonimodes</i> Drechsler	ATCC14445 (T)	JF816221	JF816226
<i>C. coronatus</i>	RCEF5598	JQ004791	JQ004795
	RCEF5599	JQ004792	JQ004796
	RCEF5600	JQ004793	JQ004797
	RCEF5601	JQ004794	JQ004798
	AFTOL-ID137	AY546691	DQ275337
<i>C. heterosporus</i> Drechsler	RCEF4430	JF816225	JF816239
<i>C. humicola</i> M.C. Sriniv. & Thirum.	ATCC28849 (T)	JF816220	JF816231
<i>C. lichenicola</i>	ATCC16200 (T)	JF816216	JF816232
<i>C. lobatus</i> M.C. Sriniv. & Thirum.	ATCC18153 (T)	JF816218	JF816233
<i>C. nodosus</i> M.C. Sriniv. & Thirum.	ATCC16577 (T)	JF816217	JF816235
<i>C. polytocus</i> Drechsler	ATCC12244 (T)	JF816213	JF816227
<i>C. stromoideus</i>	ATCC15430 (T)	JF816219	JF816229
<i>C. sinensis</i>	RCEF4952 (T)	JF816224	JF816238
<i>C. thromboides</i> Drechsler	ATCC12587 (T)	JF816214	JF816230
	RCEF4492	JF816223	JF816236
<i>E. muscae</i> (Cohn) Fresen.	ARSEF3074	DQ273772	DQ275343

* The fungal taxonomy follows that of King (1976a, b, 1977). ARSEF = ARS Entomopathogenic Fungus Collection (Ithaca, USA). ATCC = American Type Culture Collection (Manassas, USA). RCEF = Research Center for Entomogenous Fungi (Hefei, China). AFTOL-ID = Assembling the Fungal Tree of Life Identity. T = ex type.

strains of *Conidiobolus* have been deposited in the GenBank database under the access numbers shown in TABLE 1.

Sequences were aligned with Clustal X (Thompson et al. 1997). The combined data of the two loci, partial 28S rDNA and EF-1 α , were analyzed with Maximum Parsimony (MP) in PAUP* 4.0b10 (Swofford 2003), by using 1000 replicates of heuristic search of random sequence additions, branch swapping algorithm by tree bisection-reconnection (TBR) and MULTrees in effect. Gaps were treated as missing data and all characters were equally weighted. Branch support was estimated by 1000 bootstraps of 10 replicates of heuristic search with the same options as the parsimony search (Felsenstein 1985). The alignments were fed to DNAMAN software package (Version 5.2.2, Lynnon Biosoft, Canada) for calculating genetic similarities.

Results

The combined alignment of partial 28S rDNA and the EF-1 α dataset was 1468 bp in length, including 981bp from the LROR/LR5 region of 28S rDNA and 487 bp from the EF983/EF1aZ-1R region of EF-1 α . 108 sites in 28S rDNA and 24 sites in EF-1 α with ambiguous alignment were excluded from the analysis and the final alignment contained 643 parsimony-informative sites. Maximum parsimony analysis of 20-taxon dataset resulted in a single tree (TL = 1942, CI = 0.6130, RI = 0.7826, HI = 0.3553) shown in PLATE 1, and



PLATE 1. The single most parsimonious tree (TL = 1942, CI = 0.6130, RI = 0.7826, HI = 0.3553) showing phylogenetic relationships among species of *Conidiobolus* inferred from a combined dataset of partial 28S rDNA and EF-1 α sequences. Bootstrap values ≥ 50 % are labeled above relevant branches. *Entomophthora muscae* served as the outgroup. The bar at the lower left corner represents 10 changes.

TABLE 2. Similarities of partial 28S rDNA and EF-1 α sequences from *Conidiobolus* strains.*

SPECIES—STRAIN	% SIMILARITY												
	1	2	3	4	5	6	7	8	9	10	11	12	13
1 <i>C. chlamydosporus</i> —ATCC12242		99	85	85	67	67	67	65	85	85	85	85	84
2 <i>C. firmipilleus</i> —RCEF4429	98		85	85	68	68	68	66	85	85	85	85	85
3 <i>C. gonimodes</i> —ATCC14445	91	91		97	68	68	66	66	96	96	96	96	96
4 <i>C. lichenicola</i> —ATCC16200	90	89	93		68	68	66	66	96	96	96	96	96
5 <i>C. thromboides</i> —ATCC12587	73	73	75	76		99	88	86	67	68	68	68	67
6 <i>C. thromboides</i> —RCEF4492	73	73	75	76	99		88	86	67	68	68	68	66
7 <i>C. stromioideus</i> —ATCC15430	75	75	75	77	91	92		94	65	66	66	65	65
8 <i>C. sinensis</i> —RCEF4952	73	73	74	75	91	91	96		65	65	65	65	65
9 <i>C. coronatus</i> —RCEF5598	92	92	94	95	76	76	77	75		99	99	98	99
10 <i>C. coronatus</i> —RCEF5599	92	91	94	95	76	76	77	75	99		99	98	99
11 <i>C. coronatus</i> —RCEF5600	91	91	94	96	76	76	77	75	99	99		99	99
12 <i>C. coronatus</i> —RCEF5601	91	91	93	95	76	76	77	75	98	98	99		98
13 <i>C. coronatus</i> —AFTOL-ID137	91	91	94	96	76	76	77	75	99	99	100	99	

* Data refer to the overall similarities of the partial 28S rDNA (above the diagonal) and EF-1 α sequences (below the diagonal)

the DNA similarities among 13 representing strains are listed in TABLE 2. The phylogenetic tree shows that the isolate RCEF4952 clustered with the ex-type strain of *Conidiobolus stromioideus* with 100% bootstrap support (PLATE 1), but the similarities between *C. stromioideus* and *C. sinensis* were only 94% (28S rDNA) and 96% (EF-1 α) (TABLE 2). Higher intraspecific DNA similarities were measured from partial 28S rDNA and EF-1 α sequence. For example, the ranges of DNA similarities within *C. coronatus* have been found to be 98–99% (28S rDNA) and 98–100% (EF-1 α), and those within *C. thromboides* were 99% for both genes. On the other hand, the *Conidiobolus* partial 28S rDNA and EF-1 α showed high genetic divergence among species. The highest similarity of partial 28S rDNA (97%) was recorded between *C. gonimodes* and *C. lichenicola*, and the lowest (65%) between *C. coronatus* and *C. stromioideus*. The range of similarities in EF-1 α among species was 73–96%. Although the similarities between *C. chlamydosporus* and *C. firmipilleus* were 99% (28S rDNA) and 98% (EF-1 α), *C. chlamydosporus* was placed in synonymy with *C. firmipilleus* in King's classification (King 1977).

Taxonomy

Conidiobolus sinensis Y. Nie, X.Y. Liu & B. Huang, *sp. nov.*

PLATES 2–3

MYCOBANK MB563665

Differs from *Conidiobolus stromoideus* by its much longer conidiophores and rarely branching mycelia at the colony edge.

TYPE: China, Anhui Province, Huoshan County, isolated from leaf litter, 17 June 2010, [Yong Nie] (**Holotype**, RCEF4952; GenBank JF816224, JF816238).

ETYMOLOGY: *sinensis* (Lat.) = China, referring to the geographic origin of the strain.

Colonies grown on PDA for 3 days at 21°C, white, reaching ca 21 mm diameter. Numerous hyphal knots giving the colony a coarse appearance with aging. Mycelium colorless, tubular, filamentous, 5–10 µm wide, forming hyphal segments in older regions. Apical cells 80–450 µm long, often unbranched before cell division. Conidiophores colorless, unbranched and producing a single conidium, arising as upward branches from hyphal knots formed by irregular mycelium interweaving, 32.5–110 × 10–15 µm. Primary conidia colorless, globose to pyriform 17.5–25 µm wide, 22.5–32.5 µm long including a basal papilla 7.5–10 µm high and 2.5–7.5 µm wide. Primary conidia forcibly discharged, on water agar forming globose secondary conidia resembling the primary spore, 17.5–22.5 × 15–20 µm. Zygosporangia formed between adjacent conjugating cells of a hyphal body. Mature zygosporangia smooth, globose or subglobose, 25–31 µm in diameter with wall 1–2 µm thick.

Discussion

In comparing the morphological characteristics of primary conidiophores from cushion mycelium with the known *Conidiobolus* species, *C. sinensis* resembles *C. lichenicola* M.C. Sriniv. & Thirum. and *C. stromoideus* M.C. Sriniv. & Thirum. (Srinivasan & Thirumalachar 1962, 1968). Colonies of *C. lichenicola* are distinguished by a pale brownish mycelium with sinuous, lobate hyphae. *Conidiobolus stromoideus* differs from the new species producing edge mycelia that are usually branched (rarely branched in *C. sinensis*) and much shorter (12–40 µm) conidiophores (PLATE 3).

The phylogenetic tree places the *C. sinensis*–*C. stromoideus* clade distant from the *C. lichenicola* clade (PLATE 1), thus reinforcing the morphological difference between *C. sinensis* and *C. lichenicola*. If *C. chlamydosporus* is accepted as synonymous with *C. firmipilleus* in accordance with King (1977), there is a clear-cut line between intraspecific and interspecific sequence similarity levels: 98–100% within species and 64–97% among species (TABLE 2). Although *C. sinensis* groups with *C. stromoideus*, DNA similarity levels between the two species fall within the interspecific range (94% (28S rDNA) and 96% (EF-1α)). Thus, the phylogenetic analysis supports the morphological identification of *C. sinensis* as a new species differing from *C. stromoideus* and *C. lichenicola*.

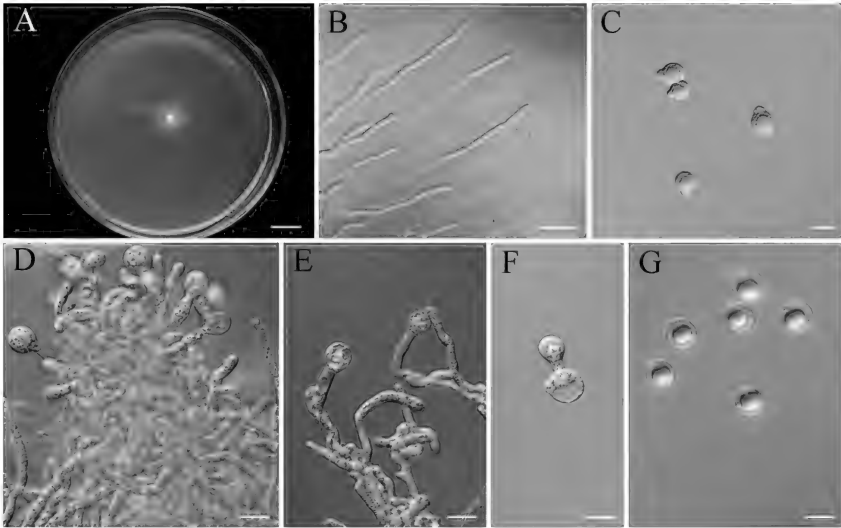


PLATE 2. *Conidiobolus sinensis*. A. Colony on PDA after 3 days at 21°C. B. Rarely branched mycelia at the margin of colony. C. Primary conidia. D. Primary conidiophores produced from hyphal knots. E. Primary conidiophores. F. Secondary conidia produced singly from the primary conidia. G. Mature zygospores. Bars: A = 10 mm, B = 100 µm, C–H = 20 µm.

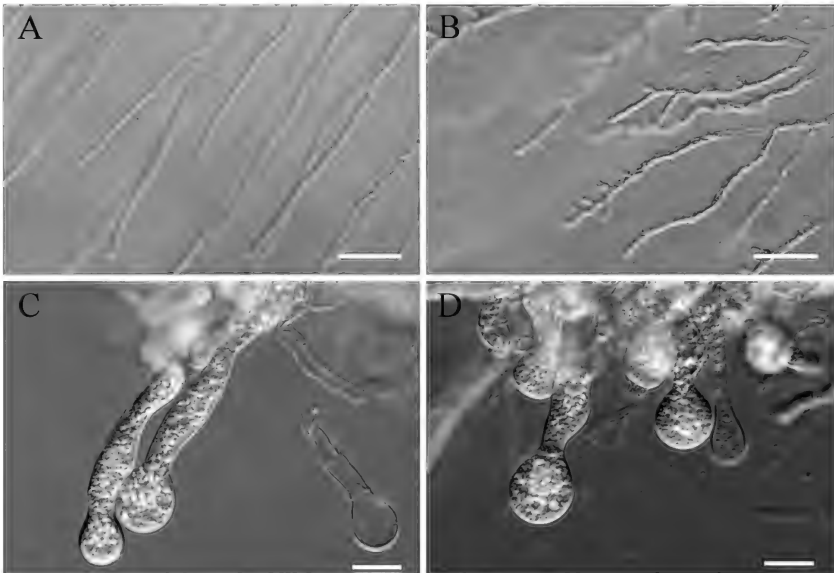


PLATE 3. A. *Conidiobolus sinensis*: rarely branched mycelia at the colony edge. B. *C. stromioideus*: moderately branched mycelia at the colony edge. C. *C. sinensis*: long conidiophores. D. *C. stromioideus*: short conidiophores. Bars: A–B = 100 µm, C–D = 20 µm.

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First record of the sequestrate fungus *Neosecotium macrosporum* (Agaricales, Lepiotaceae) from Mexico

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ABSTRACT — *Neosecotium macrosporum*, previously known only from two USA records, is reported for the first time in Mexico. Observations of macro- and microscopic characters including for the holotype and the Chihuahua collection are presented. SEM photomicrographs illustrating spore ornamentation are included.

KEY WORDS — *Basidiomycota*, taxonomy, hypogeous fungi, secotiaceous fungi

Introduction

Sequestrate fungi are considered a polyphyletic group with no evolutionary relationships whose morphological similarity comes from the adaptation to extreme habitats and environmental conditions (Albee-Scott 2007). The approximately 1200 species are distributed among eleven orders representing the phyla *Zygomycota*, *Ascomycota*, and *Basidiomycota*. Distinguishing characteristics are a fleshy, cartilaginous or elastic fruiting body with a persistent peridium that encloses the spore producing tissue and thus prevents the easy release of the spores (Kendrick 1992). Most sequestrates are mycorrhizogenic and serve as a source of food for a number of mammals (Trappe & Claridge 2003).

There are few studies of this group in Mexico. The first sequestrate fungus recorded for Mexico was collected by Lumholtz (1902) and later designated as the holotype of *Melanogaster umbrininglebus* Trappe & Guzmán. Trappe &

Guzmán (1971) described three other new hypogeous species and 13 new records for Mexico, Cázares et al. (1992) cited 17 new records, and Cázares et al. (2008) described *M. minisporus* Cázares et al. as a new species and reported the first Mexican records of three species of *Hysterangium* Vittad.

Neosecotium Singer & A.H. Sm. is a genus characterized by a dry firm whitish stipe-columella that extends below a powdery gleba containing few fibulae and strongly verrucose spores (Miller & Miller 1988). In comparison, *Secotium* Kunze is characterized by the presence of a volva, a less powdery gleba with abundant fibulae, and smooth ellipsoid large spores. *Neosecotium* is represented by two species: *N. africanum* and *N. macrosporum*.

Materials & methods

The collections were studied according to the standard mycological techniques (Cifuentes et al. 1986, Moreno & Manjón 2010). The basidia, spores, peridial hyphae, gleba, and columella of specimens mounted in Hoyer's medium were measured using a Nikon Eclipse E200 contrast phase microscope, with some samples examined using a Zeiss DSM 950 scanning electron microscope after processing in a Polaron E-2000 for 1 min at 1.2 Kv and 20 mA, in an argon atmosphere to obtain a 500 Å thick deposition of gold. The specimens are deposited in the macromycetes collection of the Centro de Estudios Superiores de Estado de Sonora (CESUES) and the Universidad Autónoma de Ciudad Juárez (UACJ). The holotype was examined using the New York Botanical Garden's Virtual Herbarium (NY). Herbarium acronyms follow Thiers (2011).

Species studied

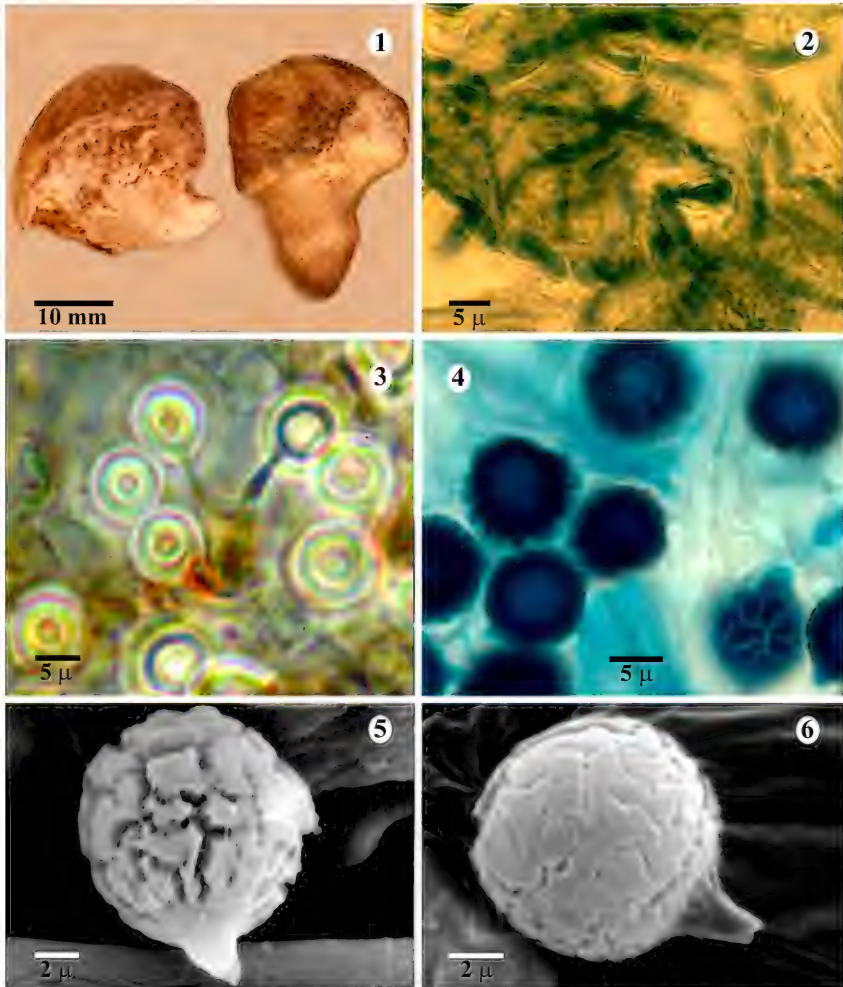
Neosecotium macrosporum (Lloyd) Singer & A.H. Sm.,

Madroño 15(5): 154 (1960).

FIGS 1–6

= *Secotium macrosporum* Lloyd Mycol. Writ. 1: 139. (1903).

BASIDIOMATA subglobose to pyriform, measuring 11–15 × 7–10 mm. PERIDIUM thick, 0.5–0.8 mm, membranaceous, yellowish brown when mature. GLEBA composed of tightly packed lamellae, compact or forming pseudo-chambers, labyrinthiform in young specimens and powdery in old specimens; yellowish and dark brown when young and mature respectively. STIPE-COLUMELLA percurrent, whitish, measuring 7–15 × 3–9 mm. PERIDIUM of septate hyphae, branched, 2.5–4 µm in diam. COLUMELLA with hyphae similar to the peridium, septate, branched, 5–7.5 µm in diam. BASIDIA clavate, (25–)28–32(–35) × 3–5 µm at the base and 9–12 µm in the middle and upper part, yellowish brown in water; evanescent, leaving amorphous remains when mature. BASIDIOSPORES globose, subglobose to ovoid, pseudoamyloid, 10–13(–14) × 8–11 µm including ornamentation, which is strongly spiny with flat distal end; episprium 2–3 µm thick, covered by a membrane, which covers the depressions between the spines, giving a cracked appearance to the wall as observed under the SEM. PEDICEL straight to slightly curved, 2–3 × 2–4 µm, narrowing toward the apex.



FIGS 1–6: *Neosecotium macrosporum* (CESUES 5600).

1. Basidiome. 2. Peridium hyphae (LM). 3. Basidium (LM). 4. Spores (LM). 5–6. Spore (SEM).

SPECIMENS EXAMINED: MEXICO, CHIHUAHUA: Municipality of Juárez, 31°13'53.1"N 106°30'36.8"W, 1120 masl, in sandy soil near *Prosopis glandulosa* Torr., 24.VII.2009, M. Lizárraga, C. Salazar, D. López-Peña, D. Sáenz, A.H. Gutiérrez, E. Navarrete (CESUES 5550); 31°17'54.35"N 106°31'55.45"W, 1303 masl, 17.IX.2010, M. Lizárraga, M. Vargas, C. Salazar, D. Sáenz (UACJ 1560, CESUES 5600); 19.III.2011, M. Vargas, M. Lizárraga (UACJ 1683); 26.III.2011 (UACJ 1684).

OBSERVATIONS— The Mexican materials were compared with the type and other authentic collections of *Neosecotium macrosporum* in the Virtual Herbarium of the New York Botanical Garden (VHNYBG 2011), which allowed us to

confirm this determination. Although the holotype (NY 809166, from Texas) is in a state of deterioration, there are two collections of authentic material (NY 809164 and 809165, from North Dakota) identified as *N. macrosporum* by S.M. Zeller.

Basidiomes of the type and authentic material, in immature stages with chambers in an obscure lamellar orientation, wood brown to cinnamon color; stipe-columella percurrent, whitish, little projection of the stipe (2–3 mm); spores globose, subglobose to ovate, 13–18 or 14–18 × 12–15 µm, pseudoamyloid, with sterigmal appendage, exosporium smooth becoming cracked into an areolate pattern and the fissures produce a warty to echinulate effect; basidia clavate to subelliptic-pedicellate, 25–37 × 14–17 µm (Lloyd 1903; Singer & Smith 1960). The Mexican collections differ in the smaller spore size compared with those previously reported. This high variability appears to be inherent to the species and has also been reported for taxa close to *N. macrosporum*.

According to descriptions by Lloyd (1903) and Singer & Smith (1960), this species is easily recognized by its stipe-columella, which extends from the base of the gleba to the apex of the peridium, lamelliform gleba and strongly verrucose pigmented spores. Although unopened fruiting bodies of *Geastrum* spp. could be confused macroscopically with *Neosecotium*, the *Geastrum* is easily distinguished by violet spores, a two-layered peridium, and capillitium. Based on similar habitat and morphology, *Neosecotium* might also be confused with *Endoptychum* Czern., which can be distinguished by its smooth spores and somewhat larger fruiting bodies (Arora 1986).

Singer & Smith (1960) regarded *Neosecotium* as intermediate between the *Agaricaceae* and *Lycoperdaceae*, based on the variation in color and spore ornamentation in some genera in both families. Castellano et al. (2004) placed *Neosecotium* into *Lepiotaceae* based on recent molecular-supported classifications.

Neosecotium macrosporum has been recorded from two localities in the United States of America (Lloyd 1903, Singer & Smith 1960); the present study represents a new record for Mexico, and the third for the world.

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**New combinations in *Lactifluus*. 3.
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ABSTRACT — In this last of a series of three papers, new combinations in the genus *Lactifluus* are proposed. This paper treats *Lactifluus* subg. *Lactifluus* (an autonymous subgenus) and *Lactifluus* subg. *Piperati* (proposed as a new subgenus). In *Lactifluus* subg. *Lactifluus*, six sections are recognized (five of them as new combinations) and 46 new combinations are proposed at species level. In *Lactifluus* subg. *Piperati*, two sections are recognized (as new combinations) and nine new species combinations are proposed. In addition, new combinations are proposed for an unassigned section and its single species as well as for three unassigned species.

KEY WORDS — milkcaps, nomenclature, *Lactarius*, *Russulaceae*

Introduction

This is the third treatment of species formerly treated in *Lactarius* Pers. and now classified in *Lactifluus* (Pers.) Roussel. *Lactifluus* subgenera *Edules*, *Lactariopsis*, and *Russulopsis* were discussed in the first article (Verbeken et al. 2012) and *Lactifluus* subg. *Gerardii* in the second (Stubbe et al. 2012).

Taxonomy***Lactifluus* subg. *Lactifluus***

This large and diverse subgenus comprises 6 sections. *Lactifluus* sect. *Rubroviolascetini*, with 2 species, is endemic to tropical Africa. Two sections, *L.* sect. *Polysphaerophori* and *L.* sect. *Pseudogymnocarpi* (each with 8 species) are almost completely African, except for one South American species in *L.* sect. *Polysphaerophori* and one Chinese species in *L.* sect. *Pseudogymnocarpi*. *L.* sect. *Phlebonemi* (11 species) and *L.* sect. *Tomentosi* (9 species) also have their major distribution in Africa, but contain some species from North and Central America, Australia, Europe and Asia. *Lactifluus* sect. *Lactifluus* with

Asian, American, and European species is the only section not represented in tropical Africa.

Five species in *Lactifluus* subg. *Lactifluus* have already been recombined: *L. corrugis*, *L. oedematopus*, *L. princeps* and *L. volemus* in *L. sect. Lactifluus*; and *L. hygrophoroides* in *L. sect. Tomentosi*.

Lactifluus* (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806, subg. *Lactifluus

TYPE: *Agaricus lactifluus* L. [= *L. volemus* (Fr. : Fr.) Kuntze] (see Buyck et al. 2010).

= *Lactarius* subg. *Lactifluus* (Burl.) Hesler & A.H. Sm., N. Am. Species *Lactarius*: 158. 1979.

TYPE: *Agaricus volemus* Fr. : Fr.

Lactifluus* (Pers.) Roussel, Fl. Calvados, Ed. 2: 66. 1806, sect. *Lactifluus

TYPE: *Agaricus lactifluus* L.

= *Lactarius* subsect. *Lactifluini* (Burl.) Singer, Ann. Mycol. 40: 114. 1942.

= *Lactarius* subsect. *Volemi* Pacioni & Lalli, Mycotaxon 44: 190. 1992, nom. superfl.

TYPE: *Agaricus volemus* Fr. : Fr.

***Lactifluus acicularis* (Van de Putte & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564580

= *Lactarius acicularis* Van de Putte & Verbeken, Fungal Diversity 45: 108. 2010.

***Lactifluus austrovolemus* (Hongo) Verbeken, comb. nov.**

MYCOBANK MB 564581

= *Lactarius austrovolemus* Hongo, Rep. Tottori Mycol. Inst. 10: 362. 1973.

***Lactifluus corrugis* (Peck) Kuntze, Revis. Gen. Pl. 2: 856. 1891.**

= *Lactarius corrugis* Peck, Annual Rep. New York State Mus. 32: 31. 1880 ("1878").

***Lactifluus crocatus* (Van de Putte & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564582

= *Lactarius crocatus* Van de Putte & Verbeken, Fungal Diversity 45: 112. 2010.

***Lactifluus distantifolius* (Van de Putte, Stubbe & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564583

= *Lactarius distantifolius* Van de Putte, Stubbe & Verbeken, Fungal Diversity 45: 115. 2010.

***Lactifluus lamprocystidiatus* (Verbeken & E. Horak) Verbeken, comb. nov.**

MYCOBANK MB 564584

= *Lactarius lamprocystidiatus* Verbeken & E. Horak, Austr. Syst. Bot. 13: 674. 2000.

***Lactifluus longipilus* (Van de Putte, H.T. Le & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564585

= *Lactarius longipilus* Van de Putte, H.T. Le & Verbeken, Fungal Diversity 45: 117. 2010.

***Lactifluus oedematopus* (Scop.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.**

= *Agaricus oedematopus* Scop., Fl. Carniol., Ed. 2, 2: 453. 1772.

***Lactifluus pallidilamellatus* (Montoya & Bandala) Van de Putte, comb. nov.**

MYCOBANK MB 564586

= *Lactarius pallidilamellatus* Montoya & Bandala, Cryptog. Mycol. 25: 16. 2004.

***Lactifluus pinguis* (Van de Putte & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564587

= *Lactarius pinguis* Van de Putte & Verbeken, Fungal Diversity 45: 119. 2010.

***Lactifluus princeps* (Berk.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.**

= *Lactarius princeps* Berk., Hooker's J. Bot. Kew Gard. Misc. 4: 135. 1852.

***Lactifluus vitellinus* (Van de Putte & Verbeken) Van de Putte, comb. nov.**

MYCOBANK MB 564588

= *Lactarius vitellinus* Van de Putte & Verbeken, Fungal Diversity 45: 121. 2010.

***Lactifluus volemus* (Fr.: Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.**

= *Agaricus volemus* Fr.: Fr., Syst. Mycol. 1: 69. 1821.

***Lactifluus* sect. *Polysphaerophori* (Singer) Verbeken, comb. nov.**

MYCOBANK MB 564589

= *Lactarius* sect. *Polysphaerophori* Singer, Beih. Sydowia 7: 106. 1973.

TYPE: *Lactarius veraecrucis* Singer.

= *Lactarius* sect. *Gymnocarpi* R. Heim ex Verbeken, Mycotaxon 66: 374. 1998.

TYPE: *Lactarius gymnocarpus* R. Heim ex Singer

***Lactifluus albocinctus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564590

= *Lactarius albocinctus* Verbeken, Syst. Geogr. Pl. 70: 182. 2000.

***Lactifluus brunnescens* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564591

= *Lactarius brunnescens* Verbeken, Bull. Jard. Bot. Belg. 65: 199. 1996.

***Lactifluus flammans* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564592

= *Lactarius flammans* Verbeken, Mycotaxon 55: 539. 1995.

***Lactifluus foetens* (Verbeken & Van Rooij) Verbeken, comb. nov.**

MYCOBANK MB 564593

= *Lactarius foetens* Verbeken & Van Rooij, Nova Hedwigia 77: 230. 2003.

***Lactifluus goossensiae* (Beeli) Verbeken, comb. nov.**

MYCOBANK MB 564594

= *Lactarius goossensiae* Beeli, Bull. Soc. Roy. Bot. Belgique 60: 165. 1928.

***Lactifluus gymnocarpus* (R. Heim ex Singer) Verbeken, comb. nov.**

MYCOBANK MB 564595

= *Lactarius gymnocarpus* R. Heim ex Singer, Pap. Michigan Acad. Sci. 32: 107. 1946.

***Lactifluus tanzanicus* (Karhula & Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564596

= *Lactarius tanzanicus* Karhula & Verbeken, Karstenia 38: 50. 1998.

***Lactifluus veraecrucis* (Singer) Verbeken, comb. nov.**

MYCOBANK MB 564597

= *Lactarius veraecrucis* Singer, Beih. Sydowia 7: 104. 1973.

***Lactifluus* sect. *Phlebonemi* (R. Heim ex Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564598

≡ *Lactarius* sect. *Phlebonemi* R. Heim ex Verbeken, Mycotaxon 66: 378. 1998.

TYPE: *Lactarius phlebonemus* R. Heim & Gooss.-Font.

= *Lactarius* subsect. *Luteoli* Pacioni & Lalli, Mycotaxon 44: 190. 1992.

≡ *Lactarius* sect. *Luteoli* (Pacioni & Lalli) Pierotti, Boll. Gruppo Micol. Bres. 48: 54. 2007.

TYPE: *Lactarius luteolus* Peck

***Lactifluus angustus* (R. Heim & Gooss.-Font.) Verbeken, comb. nov.**

MYCOBANK MB 564599

≡ *Lactarius angustus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 67. 1955.

***Lactifluus arsenei* (R. Heim) Verbeken, comb. nov.**

MYCOBANK MB 564600

≡ *Lactarius arsenei* R. Heim, Candollea 7: 380. 1938, as "*arsenii*".

***Lactifluus brunneoviolascens* (Bon) Verbeken, comb. nov.**

MYCOBANK MB 564601

≡ *Lactarius brunneoviolascens* Bon, Doc. Mycol. 1 (2): 45. 1971.

***Lactifluus caribaeus* (Pegler) Verbeken, comb. nov.**

MYCOBANK MB 564602

≡ *Lactarius caribaeus* Pegler, Kew Bull. 33: 617. 1979.

***Lactifluus longivelutinus* (X.H. Wang & Verbeken) X.H. Wang, comb. nov.**

MYCOBANK MB 564603

≡ *Lactarius longivelutinus* X.H. Wang & Verbeken, Nova Hedwigia 83 (1-2): 168. 2006.

***Lactifluus luteolus* (Peck) Verbeken, comb. nov.**

MYCOBANK MB 564604

≡ *Lactarius luteolus* Peck, Bull. Torrey Bot. Club 23: 412. 1896.

***Lactifluus nonpiscis* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564605

≡ *Lactarius nonpiscis* Verbeken, Bull. Jard. Bot. Belg. 65: 204. 1996.

***Lactifluus phlebonemus* (R. Heim & Gooss.-Font.) Verbeken, comb. nov.**

MYCOBANK MB 564606

≡ *Lactarius phlebonemus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 38. 1955.

***Lactifluus pisciodorus* (R. Heim) Verbeken, comb. nov.**

MYCOBANK MB 564607

≡ *Lactarius pisciodorus* R. Heim, Candollea 7: 380. 1938.

***Lactifluus putidus* (Pegler) Verbeken, comb. nov.**

MYCOBANK MB 564608

≡ *Lactarius putidus* Pegler, Kew Bull. 33: 620. 1979.

***Lactifluus rubrobrunnescens* (Verbeken, E. Horak & Desjardin) Verbeken, comb. nov.**

MYCOBANK MB 564609

≡ *Lactarius rubrobrunnescens* Verbeken, E. Horak & Desjardin, Sydowia 53: 274. 2001.

***Lactifluus* sect. *Pseudogymnocarpi* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564610

≡ *Lactarius* sect. *Pseudogymnocarpi* Verbeken, Mycotaxon 66: 376. 1998.

TYPE: *Lactarius gymnocarpoides* Verbeken

***Lactifluus carmineus* (Verbeken & Walley) Verbeken, comb. nov.**

MYCOBANK MB 564611

≡ *Lactarius carmineus* Verbeken & Walley, Syst. Geogr. Pl. 70: 190. 2000.

***Lactifluus gymnocarpoides* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564612

≡ *Lactarius gymnocarpoides* Verbeken, Mycotaxon 55: 530. 1995.

***Lactifluus longisporus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564613

≡ *Lactarius longisporus* Verbeken, Mycotaxon 55: 527. 1995.

***Lactifluus luteopus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564614

≡ *Lactarius luteopus* Verbeken, Mycotaxon 55: 536. 1995.

***Lactifluus medusae* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564615

≡ *Lactarius medusae* Verbeken, Mycotaxon 55: 532. 1995.

***Lactifluus pseudogymnocarpus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564616

≡ *Lactarius pseudogymnocarpus* Verbeken, Mycotaxon 55: 523. 1995.

***Lactifluus pumilus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564617

≡ *Lactarius pumilus* Verbeken, Bull. Jard. Bot. Belg. 65: 205. 1996.

***Lactifluus tenuicystidiatus* (X.H. Wang & Verbeken) X.H. Wang, comb. nov.**

MYCOBANK MB 564618

≡ *Lactarius tenuicystidiatus* X.H. Wang & Verbeken, Nova Hedwigia 83: 173. 2006.

***Lactifluus* sect. *Rubroviolascetini* (Singer) Verbeken, comb. nov.**

MYCOBANK MB 564619

≡ *Lactarius* subsect. *Rubroviolascetini* Singer, Ann. Mycol. 40: 114. 1942.

≡ *Lactarius* sect. *Rubroviolascetini* (Singer) Verbeken,

Mycotaxon 66: 380. 1998, as "*Rubroviolascetes*".

TYPE: *Lactarius rubroviolascens* R. Heim

***Lactifluus denigricans* (Verbeken & Karhula) Verbeken, comb. nov.**

MYCOBANK MB 564620

≡ *Lactarius denigricans* Verbeken & Karhula, Persoonia 16: 219. 1996.

***Lactifluus rubroviolascens* (R. Heim) Verbeken, comb. nov.**

MYCOBANK MB 564621

≡ *Lactarius rubroviolascens* R. Heim, Candollea 7: 377. 1938.

***Lactifluus* sect. *Tomentosi* (McNabb) Verbeken, comb. nov.**

MYCOBANK MB 564622

= *Lactarius* sect. *Tomentosi* McNabb, New Zealand J. Bot. 9: 59. 1971.

= *Lactarius* subsect. *Clarkeina* McNabb, New Zealand J. Bot. 9: 59. 1971.

TYPE: *Lactarius clarkeae* Cleland

= *Lactarius* subsect. *Rugati* Pacioni & Lalli, Mycotaxon 44: 190. 1992, nom. superfl.

= *Lactarius* sect. *Rugati* Verbeken, Mycotaxon 66: 372. 998, 1998, nom. superfl.

TYPE: *Lactarius rugatus* Kühner & Romagn.

***Lactifluus clarkeae* (Cleland) Verbeken, comb. nov.**

MYCOBANK MB 564623

= *Lactarius clarkeae* Cleland, Trans. & Proc. Roy. Soc.

S. Australia 51: 302. 1927, as "*clarkei*".

***Lactifluus hygrophoroides* (Berk. & M.A. Curtis) Kuntze, Revis. Gen. Pl. 2: 857. 1891.**

= *Lactarius hygrophoroides* Berk. & M.A. Curtis, Ann. Mag. Nat. Hist., Ser. 3, 4: 293. 1859.

***Lactifluus kivuensis* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564624

= *Lactarius kivuensis* Verbeken, Bull. Jard. Bot. Belg. 65: 202. 1996.

***Lactifluus pseudoluteopus* (X.H. Wang & Verbeken) X.H. Wang, comb. nov.**

MYCOBANK MB 564625

= *Lactarius pseudoluteopus* X.H. Wang & Verbeken, Nova Hedwigia 83: 171. 2006.

***Lactifluus pseudovolemus* (R. Heim) Verbeken, comb. nov.**

MYCOBANK MB 564626

= *Lactarius pseudovolemus* R. Heim, Candollea 7: 378. 1938.

***Lactifluus rubiginosus* (Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564627

= *Lactarius rubiginosus* Verbeken, Bull. Jard. Bot. Belg. 65: 207. 1996.

***Lactifluus rugatus* (Kühner & Romagn.) Verbeken, comb. nov.**

MYCOBANK MB 564628

= *Lactarius rugatus* Kühner & Romagn., Bull. Soc. Mycol. France 69: 362. 1954 ("1953").

***Lactifluus xerampelinus* (Karhula & Verbeken) Verbeken, comb. nov.**

MYCOBANK MB 564629

= *Lactarius xerampelinus* Karhula & Verbeken, Karstenia 38: 59. 1998.

***Lactifluus volemoides* (Karhula) Verbeken, comb. nov.**

MYCOBANK MB 564630

= *Lactarius volemoides* Karhula, Karstenia 38: 53. 1998.

Lactifluus* subg. *Piperati

This group consists of two sections, one with 9 species described from Europe and Asia (but also with records from North America), and the other with one American species. The combination *Lactifluus piperatus* has already been proposed.

***Lactifluus* subg. *Piperati* Verbeken, subg. nov.**

MYCOBANK MB 564631

Pileus pallidus, saepe albus vel albidus, siccus. Pileipellis hyphoepithelium, tenue stratum hyphis hyalinis super cellulis globosis. Dermatocystidia interdum presentia.

TYPE: *Agaricus piperatus* L.: Fr.

***Lactifluus* sect. *Piperati* (Fr.) Verbeken, comb. nov.**

MYCOBANK MB 564632

≡ *Agaricus* sect. *Piperati* Fr., Syst. Mycol. 1: 73. 1821.

≡ *Lactarius* sect. *Piperati* (Fr.: Fr.) Fr., Epicr. Syst. Mycol.: 338. 1838.

TYPE: *Agaricus piperatus* L.: Fr.

***Lactifluus dwaliensis* (K. Das, J.R. Sharma & Verbeken) K. Das, comb. nov.**

MYCOBANK MB 564633

≡ *Lactarius dwaliensis* K. Das, J.R. Sharma & Verbeken, Mycotaxon 88: 334. 2003.

***Lactifluus glaucescens* (Crossl.) Verbeken, comb. nov.**

MYCOBANK MB 564634

≡ *Lactarius glaucescens* Crossl., Naturalist, J. Nat. Hist. N. England 1900(516): 5. 1900.

≡ *Lactarius piperatus* var. *glaucescens* (Crossl.) Hesler & A.H.

Sm., N. Amer. Species *Lactarius*: 186. 1979

***Lactifluus leucophaeus* (Verbeken & E. Horak) Verbeken, comb. nov.**

MYCOBANK MB 564635

≡ *Lactarius leucophaeus* Verbeken & E. Horak, Austr. Syst. Bot. 12: 768. 1999.

***Lactifluus novoguineensis* (Henn.) Verbeken, comb. nov.**

MYCOBANK MB 564636

≡ *Lactarius novoguineensis* Henn., Bot. Jahrb. Syst. 25: 503. 1898.

***Lactifluus olivescens* (Verbeken & E. Horak) Verbeken, comb. nov.**

MYCOBANK MB 564637

≡ *Lactarius olivescens* Verbeken & E. Horak, Austr. Syst. Bot. 13: 678. 2000.

***Lactifluus paleus* (Verbeken & E. Horak) Verbeken, comb. nov.**

MYCOBANK MB 564638

≡ *Lactarius paleus* Verbeken & E. Horak, Austr. Syst. Bot. 12: 771. 1999.

***Lactifluus piperatus* (L. : Fr.) Kuntze, Revis. Gen. Pl. 2: 857. 1891.**

≡ *Agaricus piperatus* L.: Fr., Sp. Pl.: 1173. 1753.

≡ *Lactarius piperatus* (L. : Fr.) Pers., Tent. Disp. Meth. Fung.: 64. 1797.

≡ *Galorrheus piperatus* (L.: Fr.) Fr., Stirp. Agri Femsion. 3: 57. 1825.

***Lactifluus roseophyllus* (R. Heim) De Crop, comb. nov.**

MYCOBANK MB 564639

≡ *Lactarius roseophyllus* R. Heim, Rev. Mycol. (Paris) 30: 237. 1966 ("1965").

***Lactifluus subpiperatus* (Hongo) Verbeken, comb. nov.**

MYCOBANK MB 564647

≡ *Lactarius subpiperatus* Hongo, Mem. Fac. Liberal Arts Shiga Univ., Nat. Sci. 15: 46. 1964.

Lactifluus* sect. *Allardii (Hesler & A.H. Sm.) De Crop, **comb. nov.**

MYCOBANK MB 564640

≡ *Lactarius* sect. *Allardii* Hesler & A.H. Sm., N. Amer. Species *Lactarius*: 207. 1979.

TYPE: *Lactarius allardii* Coker

Lactifluus allardii (Coker) De Crop, **comb. nov.**

MYCOBANK MB 564641

≡ *Lactarius allardii* Coker, J. Elisha Mitchell Sci. Soc. 34: 12. 1918.

Unassigned taxa

The following section has not yet been assigned to a subgenus. Only one species, described from tropical Africa, is known.

Lactifluus* sect. *Aurantiifolii (Verbeken) Verbeken, **comb. nov.**

MYCOBANK MB 564642

≡ *Lactarius* sect. *Aurantiifolii* Verbeken, Mycotaxon 77: 441. 2001.

TYPE: *Lactarius aurantiifolius* Verbeken

Lactifluus aurantiifolius (Verbeken) Verbeken, **comb. nov.**

MYCOBANK MB 564643

≡ *Lactarius aurantiifolius* Verbeken, Bull. Jard. Bot. Belg. 65: 197. 1996.

The following species have an uncertain systematic position, but morphological and/or molecular data support their placement in *Lactifluus*.

Lactifluus caperatus (R. Heim & Gooss.-Font.) Verbeken, **comb. nov.**

MYCOBANK MB 564644

≡ *Lactarius caperatus* R. Heim & Gooss.-Font., Bull. Jard. Bot. État 25: 36. 1955.

Lactifluus cocosmus (Van de Putte & De Kesel) Van de Putte, **comb. nov.**

MYCOBANK MB 564645

≡ *Lactarius cocosmus* Van de Putte & De Kesel, Cryptog. Mycol. 30: 40. 2009.

Lactifluus subclarkeae (Grgur.) Verbeken, **comb. nov.**

MYCOBANK MB 564646

≡ *Lactarius subclarkeae* Grgur., Larger Fungi S. Australia: 63, 1997.

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<http://dx.doi.org/10.5248/120.451>

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***Exobasidium ferrugineae* sp. nov., associated with hypertrophied flowers of *Lyonia ferruginea* in the southeastern USA**AARON H. KENNEDY¹, NISSE A. GOLDBERG² & ANDREW M. MINNIS^{3*}¹National Identification Services, USDA-APHIS-PPQ-PHP,
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ABSTRACT — *Exobasidium ferrugineae*, associated with hypertrophied flowers and less commonly leaves of *Lyonia ferruginea* (rusty staggerbush), is formally described here as a new species. Morphological and DNA sequence (ITS, nLSU) data are provided. Phylogenetic analyses confirm that it is not conspecific with any species of *Exobasidium* represented by existing DNA sequence data. A key to North American species of *Exobasidium* on *Lyonia* is presented.

KEY WORDS — Basidiomycota, Ericaceae, Exobasidiales, Exobasidiomycetes, plant pathogen

Introduction

Exobasidium Woronin (Exobasidiales, Exobasidiomycetes) is a basidiomycetous genus associated with diseases of ericaceous plants commonly characterized by formation of galls on leaves, shoots, and flowers (Burt 1915, Savile 1959, Nannfeldt 1981). Early authors named species on the basis of symptomatology and host association, whereas monographers, including Burt (1915) and Savile (1959), advocated broader taxonomic concepts. These authors suggested that symptoms were variable, overlapping, and dependent on time and environmental conditions. Furthermore, fungal morphology was not definitive for species recognition and usually poorly known, and host associations are not supported by inoculation and cross-inoculation experiments. According to these authorities, only a small number of species were accepted in *Exobasidium* and the citation of numerous synonyms was deemed unreliable (Burt 1915, Savile 1959). Nannfeldt (1981) reviewed a large amount of data and literature

with special emphasis on life cycles and symptoms. He concluded that species of *Exobasidium*, though often quite similar morphologically, are rigidly host-limited taxa. Recent molecular studies including those by Begerow et al. (2002) and Piątek et al. (2012) support Nannfeldt's hypotheses on the importance of host association and host specificity.

In April 2011, an unusual flower of *Lyonia ferruginea* (Walter) Nutt. (rusty staggerbush; *Ericaceae*) that was infected and highly transformed by a fungus was found in Florida. Microscopic examination confirmed that a species of *Exobasidium* was growing on the hypertrophied flower. This species was subsequently characterized with morphological and DNA sequence (ITS, nLSU) data. Careful comparison with known species using historical literature, herbarium specimens, and phylogenetic analyses revealed that it represents an undescribed taxon that is described herein. A key to North American species of *Exobasidium* on *Lyonia* is presented in order to facilitate identification and promote further study of *Exobasidium* from North America.

Materials & methods

Morphology

Observations and measurements of microscopic characters were made from crush mounts of material scraped from the hymenial surface that covered the flower. Material was rehydrated and viewed in 3% KOH, and occasionally phloxine solution was used to improve the visibility of fungal structures (Largent et al. 1977). Sterigmata are not included in length measurements of basidia. Voucher specimens were deposited into the U.S. National Fungus Collections (BPI).

Molecular data collection & phylogenetic analysis

DNA was extracted from scraped hymenial surface material from the infected flower of *L. ferruginea* (N.A. Goldberg, s.n.; BPI 882571) using Qiagen's DNeasy Plant Mini Kit (Germantown, MD). Ribosomal DNA sequence data were generated from the internal transcribed spacer region (ITS; ITS1, 5.8S, ITS2) and the nuclear encoded large subunit (nLSU) using the primer pairs ITS5/ITS4 (White et al. 1990) and NL1/NL4 (O'Donnell 1992), respectively. GoTaq (Promega, Madison, WI) and associated standard reagents were used for PCR following the manufacturer recommendations including 2.0 mM MgCl. Standard thermal cycling was used including an annealing temperature of 55°C for the ITS and 50°C for the nLSU. PCR products were fluorescently labeled using respective forward and reverse PCR primers and the BigDye v3.1 dye terminator kit (Applied Biosystems; Foster City, CA). The purified products were then sequenced on an ABI 3730 automated DNA sequencer. Electropherograms were edited in Geneious Pro v.5 (Drummond et al. 2010). Resulting consensus sequences were submitted to GenBank (<http://www.ncbi.nlm.nih.gov>).

BLAST searches were conducted independently with the ITS and nLSU sequences against GenBank using the megablast algorithm. Taxon sampling for phylogenetic analyses was primarily determined by selecting the top hits from each search, sorted by Max Score; the phylogenetic results of Piątek et al. (2012); and morphological and

host association similarity. The ITS and nLSU sequences for each taxonomic unit were derived from the same isolate.

Multiple sequence alignments of the ITS and nLSU were conducted using MUSCLE v3.6 (Edgar 2004) within Geneious Pro v5 (Drummond et al. 2010) and then concatenated. Although nLSU data were available for all taxa included in the concatenated alignment, ITS data were unavailable for *Exobasidium karstenii*, *E. oxycocci*, *E. pieridis*, and *E. sundstroemii* and therefore treated as missing data. The best-fit model of DNA sequence evolution was independently determined for ITS and nLSU in MrModeltest v2.2 (Nylander 2004) according to the Akaike Information Criterion (AIC; Posada & Buckley 2004). The GTR+I+G model was selected for both data sets and implemented in the following analysis. The concatenated alignment was analyzed phylogenetically with Bayesian Inference (BI) in MrBayes v3.1.2 (Huelsenbeck & Ronquist 2001; Ronquist & Huelsenbeck 2003) leaving all other parameters as default. The posterior probability (pp) distribution of trees was estimated from two independent Markov Chain Monte Carlo (MCMC) simulations of 3 million generations, sampling trees every 100 generations until the standard deviation of split frequencies reached 0.01. The burn-in was determined using the program Tracer v1.5 (Rambaut & Drummond 2007). A 50% majority rule consensus tree was constructed from the remaining trees in FigTree v1.3.1 (Rambaut 2009).

The concatenated alignment was also analyzed using Maximum Likelihood (ML). This analysis was conducted using RAxML (Stamatakis 2006) via the on-line CIPRES (Cyberinfrastructure for Phylogenetic Research) Science Gateway V. 3.1 (http://www.phylo.org/sub_sections/portal/; Miller et al. 2010) with the RAxML-HPC BlackBox (7.2.8) tool. One thousand bootstrap (bs) replicates were run and all other parameters were left as default.

Results

Alignment of the ITS resulted in a matrix of 595 nucleotide positions, 296 of which were variable (49.7%) and 156 of which were parsimony informative (26.2%). Alignment of the nLSU resulted in a matrix of 555 nucleotide positions, 146 of which were variable (26.3%) and 71 of which were parsimony informative (12.8%).

The phylogenies resulting from the BI and ML analyses were entirely congruent with the exception of the *E. pieridis*/*E. ferrugineae* clade, which occupied a different position in the most optimal ML tree vs. the BI consensus tree (Fig. 1). This is an insignificant observation considering the position of this clade is entirely unsupported in the ML bootstrap tree and received very low posterior probability in the BI tree. Each analysis revealed high support (pp = 0.99, bs = 99%) for the *E. pieridis*/*E. ferrugineae* clade, revealing a close relationship between these species and demonstrating that *E. ferrugineae* is a species distinct from *E. vaccinii* (Fig. 1).

The relationship between *Exobasidium ferrugineae* and *E. karstenii* was of particular interest because *E. karstenii* was among the top hits for nLSU.

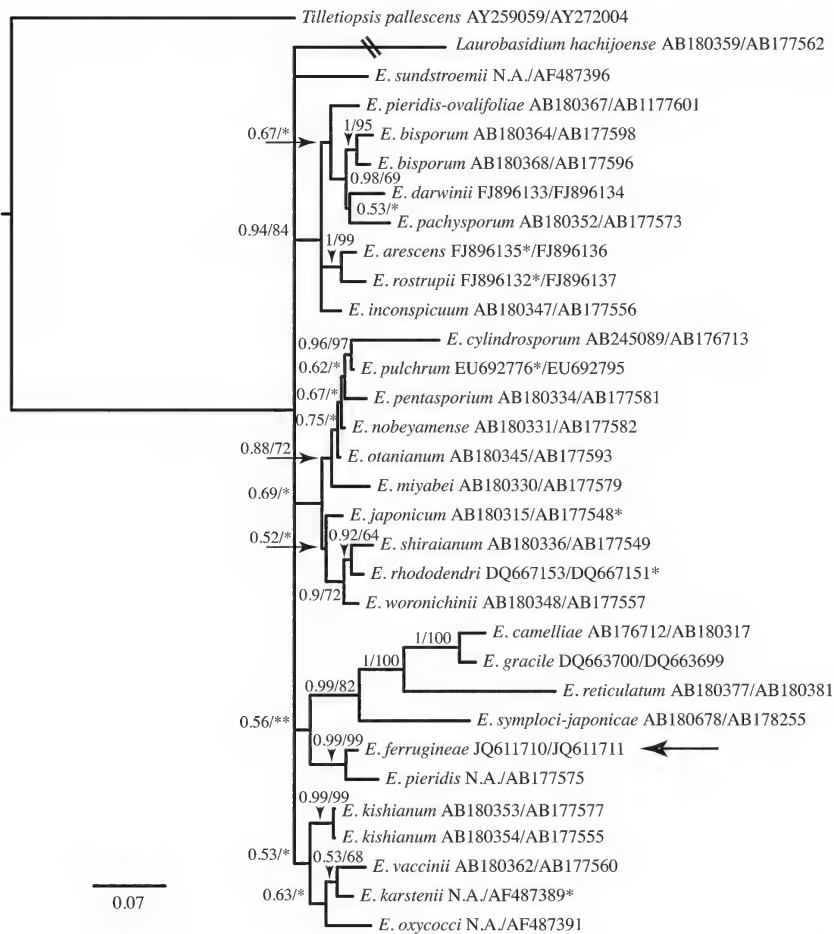


FIG. 1. A phylogenetic summary of relationships among *Exobasidium* species resulting from Bayesian Inference (BI) and Maximum Likelihood (ML) analyses of a combined ITS/nLSU data matrix. This phylogram is the resulting 50% majority rule consensus tree from the BI analysis with corresponding posterior probabilities (pp) and appended bootstrap (bs) values from the ML analysis on each branch (pp/bs). One asterisk indicates a bs value < 50%, while two asterisks indicates a clade that was not recovered in the most optimal ML tree. The hatch marks on the "*Laurobasidium hachijoense*" nom. prov. in GenBank [= *E. hachijoense*] branch indicate that it was shortened for presentation purposes from 8.5 times the scale bar. GenBank accession numbers are provided after each taxon name (ITS/nLSU). Asterisks here indicate top BLAST hits for the associated marker. The arrow indicates the position of *E. ferrugineae* (holotype, BPI 882571).

However, our phylogenetic results suggested, albeit with very weak support (pp=0.53, bs = 68), that *E. karstenii* is most closely related to *E. vaccinii*. Again, the relationship of this clade to others was unsupported.

Taxonomy

Exobasidium ferrugineae Minnis, A.H. Kenn. & N.A. Goldberg, sp. nov. FIGS. 2–4

MYCOBANK 564773

Characterized by its association with hypertrophied flowers of *Lyonia ferruginea*.

TYPE: USA. Florida: Jacksonville, Jacksonville Arboretum, Rosemary Ridge Trail, on flower of *Lyonia ferruginea*, 18.IV.2011, leg. N.A. Goldberg, s.n., (Holotype BPI 882571; GenBank accession nos., ITS JQ611710, nLSU JQ611711).

ETYMOLOGY: The species epithet is derived from the host, *Lyonia ferruginea*.

HYMENIUM consisting of a thin white layer on hypertrophied flowers having all parts proportionally enlarged, infected flowers up to approx. 6 cm long, or less commonly on leaves that may also be hypertrophied. BASIDIA 37–54.5 × 6.5–8.5 µm, cylindrical to cylindrical-clavate, 3–5 sterigmate, thin walled, hyaline, often with granular contents. STERIGMATA approx. 4.5–6.5 × 1–2 µm, typically slightly wider towards base. BASIDIOSPORES 13.5–19 × 4–6.5 µm, ellipsoid to musiform, with a prominent apiculus, walls thin and smooth, initially with one more or less median septum at maturity, later with up to six septa, hyaline. GERMINATION via germ tubes at both ends of basidiospores. CONIDIA 4–9.5 × 1–2.5 µm, bacilliform, ellipsoid or slightly clavate, walls thin and smooth, aseptate, hyaline, contents often with a few scattered guttules.

HABITAT & DISTRIBUTION— This species is known from hypertrophied flowers and less commonly hypertrophied leaves of *Lyonia ferruginea* (*Ericaceae*). It is known from Florida and Georgia, and is predicted to occur in South Carolina, and throughout the narrow, native range of its host (USDA 2011).

ADDITIONAL SPECIMENS EXAMINED— USA. FLORIDA: on flower of *Lyonia ferruginea*, leg. Chapman, s.n., Mo. Bot. Gard. Herb. 44409 (BPI 292003 as *Exobasidium vaccinii*); Dunedin, on flower of *Lyonia ferruginea*, 16.IV.1900, leg. S.M. Tracy, SMT 7159, Mo. Bot. Gard. Herb. 4962 (BPI 292004 as *E. vaccinii*); Jacksonville, Jacksonville Arboretum, Rosemary Ridge Trail, on leaves of *Lyonia ferruginea*, 06.V.2011, leg. N.A. Goldberg, s.n. (BPI 882572); additional collections on *Lyonia ferruginea* made by N.A. Goldberg from the area of the type locality, Rosemary Ridge Trail: 18.IV.2011 (BPI 882566, BPI 882567, BPI 882568, BPI 882569, BPI 882570, BPI 882580, BPI 882582, BPI 882583), 06.V.2011 (BPI 882573, BPI 882574, BPI 882575, BPI 882576, BPI 882577, BPI 882578, BPI 882579, BPI 882581). GEORGIA: Brunswick, on flower of *Lyonia ferruginea*, 01.VI.1901, leg. W. Trelease, s.n., Mo. Bot. Gard. Herb. 4955 (BPI 292005 as *E. vaccinii*).

COMMENTS— Burt (1915) studied collections of hypertrophied flowers of *Lyonia ferruginea* caused by *Exobasidium ferrugineae* and concluded that the species was conspecific with *E. vaccinii*. Burt (1915) also commented on the fact that the hypertrophied flowers were much larger than those of *L. mariana* found in association with *E. peckii* Halst., which he also concluded was a synonym of *E. vaccinii*. However, the results presented here show that *E. ferrugineae* is distinct from *E. vaccinii*. *Exobasidium vaccinii* has been shown to be restricted



FIG. 2. *Exobasidium ferrugineae* associated with hypertrophied flowers of *Lyonia ferruginea*. Note uninfected flowers in upper image for comparison. All flower parts are proportionally enlarged.

to *Vaccinium*, likely just *Vaccinium vitis-idaea* (Nannfeldt 1981, Begerow et al. 2002, Piątek et al. 2012).



FIG. 3. *Exobasidium ferrugineae* associated with hypertrophied leaves of *Lyonia ferruginea*.

Several species of *Exobasidium* are known from *Lyonia* (Farr & Rossman 2011) and eight of these were described originally from this host genus. Five Asian species, *E. kunmingense* Zhen Ying Li & L. Guo (Li & Guo 2009), *E. lyoniae* Zhen Ying Li & L. Guo (Li & Guo 2006), *E. ovalifoliae* Zhen Ying Li & L. Guo (Li & Guo 2008), *E. pieridis* Henn. (Hennings 1903), and *E. pieridis-ovalifoliae* Sawada (Sawada 1931), are distinguished from *E. ferrugineae* by their occurrence on *L. ovalifolia* and their association that is confined to leaf diseases. There are three previously described species on *Lyonia* from North America. *Exobasidium andromedae* Peck (Peck 1874) and *E. fawcettii* Massee (Massee 1908) occur on different hosts than *E. ferrugineae* and are associated with leaf diseases. At times, *E. andromedae* may produce unique bag galls where developing leaves are transformed into hollow bags (Peck 1874, Burt 1915). *Exobasidium peckii* is similar to *E. ferrugineae* as it infects both flowers and leaves (Halstead 1893, Stewart 1896), but it is distinguished by its occurrence on *Lyonia mariana* and a lesser amount of hypertrophy on flowers. Based on our morphological examination of a collection of *E. peckii* (USA: New York, Hicksville, Long Island, on flower of *Lyonia mariana*, 25.VI.1919, leg. G.P. Clinton, s.n. (BPI 292023 as *E. vaccinii*)), this species is nearly indistinguishable morphologically from *E. ferrugineae*. However, *E. peckii* differs in the basidiospores with none having been observed with more than 5 septa along with typically fewer septa per basidiospore and the conidia having a tendency towards an allantoid shape.

Our phylogenetic analyses indicate that *E. ferrugineae* is distinct from all species with existing ITS and nLSU sequence data in GenBank (data were



FIG. 4. *Exobasidium ferrugineae* (holotype, BPI 882571).
A–B. Basidia. C. Basidiospore. D. Conidia. Scale bars are 20 μ m for A–B, 10 μ m for C–D.

unavailable for species on *Lyonia* including *E. andromedae*, *E. fawcettii*, *E. kunmingense*, *E. lyoniae*, *E. ovalifoliae*, *E. peckii*) including the isolates that are labeled *E. pieridis* and *E. pieridis-ovalifoliae* from *Lyonia neziki*. The close relationship between *E. ferrugineae* and *E. pieridis* is noteworthy because of their highly discontinuous geographic ranges. We suggest that this geographic isolation has led to reproductive isolation and that the relationship between *E. ferrugineae* and *E. pieridis* is a product of the tendency for host specificity in *Exobasidium*, in this case with *Lyonia*, and divergence in host range relative to their recent common ancestor. Therefore, their well-supported sister-relationship found here suggests recent common ancestry, but not conspecificity. Unfortunately, ITS data were unavailable for *E. pieridis*, resulting in missing data for combined analysis. This is significant because the ITS region is more informative than nLSU primarily because it more rapidly accumulates substitutions among species. This is evidenced by the fact that the

ITS region contains twice the number of variable and informative characters among *Exobasidium* species. Therefore, support for this relationship may be artificially high in this combined analysis due to missing ITS data for *E. pieridis*. Further sampling will be needed to address the complex patterns of speciation including potential co-speciation in *Exobasidium* as discussed by Begerow et al. (2002) and Piątek et al. (2012).

Key to *Exobasidium* on *Lyonia* of North America

1. On *L. ligustrina*, with bag galls *E. andromedae*
1. On other species of *Lyonia*, bag galls absent 2
2. On *L. jamaicensis*, with hypertrophied leaves *E. fawcettii*
2. On other species of *Lyonia*, associated with flowers & occasionally leaves 3
3. On *L. mariana*, with hypertrophied flowers relatively small (up to approx.
2 × longer), some conidia allantoid *E. peckii*
3. On *L. ferruginea*, with hypertrophied flowers relatively large (up to approx.
5 × longer), conidia not allantoid *E. ferrugineae*

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MYCOTAXON

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***Passalora aseptata*, a new cercosporoid fungus
from northeastern Uttar Pradesh, India**RAGHVENDRA SINGH*, BALMUKUND CHAURASIA,
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ABSTRACT — The anamorphic fungus *Passalora aseptata* sp. nov., found on *Terminalia bellirica* (Combretaceae), in the Chowk Forest, Mahrajanj, U.P., India, is described and illustrated.

KEY WORDS — biodiversity, foliar diseases, phytopathogenic fungi, taxonomy

Introduction

During an investigation of plant diseases, fungi were collected on living leaves from forests in northeastern Uttar Pradesh during 2008. Among the collections an undescribed cercosporoid species was found. Based on pigmented conidia and conidiophores as well as conidiogenous cells with thickened scars, this fungus can be assigned to *Passalora* as circumscribed by Crous & Braun (2003).

Materials & methods

Specimens with disease symptoms of cercosporoid fungi on living leaves were collected during the course of field trips. Detailed observations of morphological characters were carried out by means of an Olympus CX31 light microscope using oil immersion (1000×). Specimens for microscopic observation were prepared by hand sectioning. Water and lactophenol were used as mounting media. Measurements were made of 30 conidia, hila, and conidiophores and of 15 stromata. Line drawings were prepared at a magnification of 1000×. Morphotaxonomic determinations were made with the help of current literature and available resident expertise. The holotype has been deposited in Herbarium Cryptogamiae Indiae Orientalis (HCIO), Indian Agricultural Research Institute, New Delhi, India; and an isotype was retained in the herbarium of Department of Botany, D.D.U. Gorakhpur University (GPU) for further reference.

Taxonomy

Passalora aseptata R. Singh, Chaurasia, K. Shukla & Upadhyaya, **sp. nov.**

MYCOBANK MB 519057

FIG. 1

Differs from all other *Passalora* species in aseptate conidia and conidiophores.

TYPE: India, Uttar Pradesh, Mahrajganj, Chowk Forest, on living leaves of *Terminalia belirica* (Gaertn.) Roxb. (*Combretaceae*), Jan. 2008, coll. Raghvendra Singh, (**holotype**, HCIO 48785; **isotype**, GPU KSR-395).

ETYMOLOGY: Latin, *aseptata* refers to aseptate conidia and conidiophores.

Infection spots hypogenous, discrete and initially vein-limited but later coalescing to become irregular and more or less necrotic, light brown, spreading over entire lower leaf surface. Colonies hypophyllous, effuse, velvety, light brown. Mycelium internal. Stromata well developed, erumpent, pseudoparenchymatous, substomatal, $26\text{--}43 \times 22\text{--}31 \mu\text{m}$. Conidiophores arising from stromata, in fascicles of 10–12, macronematous, simple, erect to procumbent, straight to flexuous, cylindrical, unbranched, smooth, thin-walled,

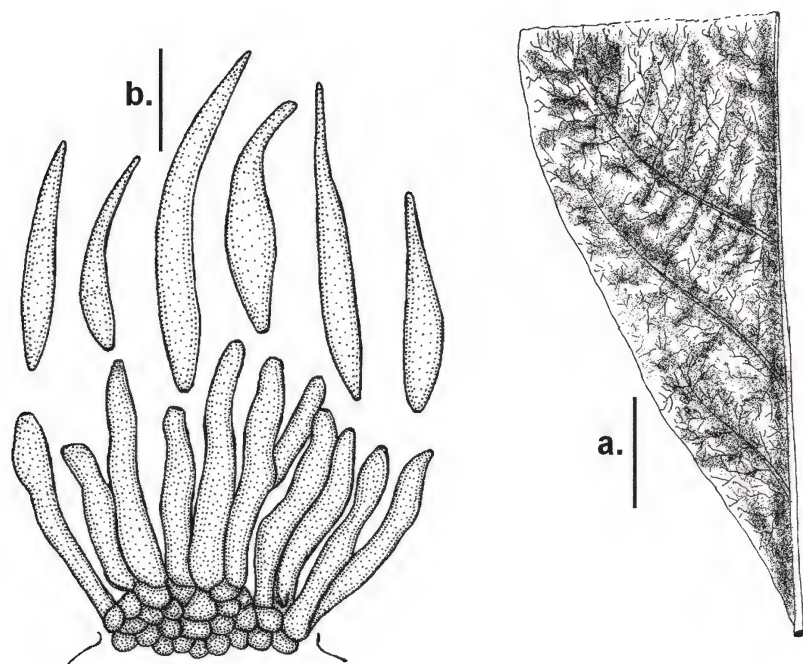


FIG. 1. *Passalora aseptata* (holotype).
a: symptoms; b: stroma, conidia, and conidiophores.
(Scale bars: a = 20 mm, b = 20 μm).

light brown to olivaceous-brown, aseptate, $29\text{--}48 \times 4\text{--}7\ \mu\text{m}$. Conidiogenous cells integrated, terminal, monoblastic, scars conspicuously thickened, darkened, $0.7\text{--}1.5\ \mu\text{m}$ wide. Conidia solitary, dry, acropleurogenous, simple, obclavate-cylindrical, erect, straight to curved, sometimes rostrate, aseptate, thin-walled, smooth, light brown to olivaceous-brown, $40\text{--}70 \times 4\text{--}9\ \mu\text{m}$, hila slightly thickened, $0.7\text{--}1.5\ \mu\text{m}$ wide.

REMARKS — No species of *Passalora* Fr. has previously been described with aseptate conidia and conidiophores. A survey of literature also indicates that no species of *Passalora* has previously been described on a host of genus *Terminalia* or family *Combretaceae*.

Prathigada terminaliae (Syd.) B. Sutton (Sutton 1994), *Pseudocercospora brevis* B. Sutton (Sutton 1994), *P. combretacearum* var. *minima* B. Sutton (Sutton 1994), *P. combreti* A.K. Singh & Kamal (Singh & Kamal 1987), and *Scolecostigmina combreti* (J. Kranz) U. Braun (Braun 1999) have been reported on the same host species. *Prathigada terminaliae* is different due to the presence of polyblastic, cicatrized, conidiogenous cells with thin, flat conidiogenous loci and rostrate conidia having smooth or rugulose, multiseptate (septa often rather thick and dark) and unequally pigmented cells. All *Pseudocercospora* species differ from *Passalora aseptata* in having euseptate conidia and conidiogenous cells without thickened scars. *Scolecostigmina combreti* is readily separated by its formation of sporodochia, annellations on conidiophores and truncate base of conidia.

Passalora bacilligera (Mont. & Fr.) Mont. & Fr., type species of *Passalora*, and all other species of this genus are easily distinguished from the novel species in having septate conidia.

Acknowledgments

The authors are grateful to Prof. Dr. Uwe Braun and Dr. Eric H.C. McKenzie for reviewing the manuscript. We also express our deep thanks to Dr. Shaun Pennycook for nomenclatural review. We are much obliged to Prof. Dr. Kamal for the identification of host plant and editing the manuscript. Authors' thanks are also due to the Curator, HCIO, New Delhi, for accepting the holotype specimens and providing an accession number thereof.

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First records of *Craterium aureonucleatum*, *Perichaena quadrata*, and *Physarum mutabile* in Italy

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ABSTRACT — Three species of *Mycetozoa*, new for Italy, were identified in garrigues and maquis ecosystems on the volcanic island of Pantelleria (Sicily). The finding of *Craterium aureonucleatum*, *Perichaena quadrata*, and *Physarum mutabile* widens their distribution in Europe and identifies new substrata.

KEY WORDS — biodiversity, Mediterranean area, ecology

Introduction

Within a wide-scoped biodiversity census, field investigations were carried out on the island of Pantelleria (Trapani province, southwest Sicily, Italy). In his checklist of *Mycetozoa* in the Mediterranean area, Lado (1994) reported 421 taxa, of which 164 were recorded from Italy. To increase the knowledge of mycetozoan diversity in Italy, we focused primarily on the collection of *Mycetozoa* in Mediterranean forest ecosystems characterizing the plant landscape of Pantelleria (Gianguzzi 1999). The *Erico arboreae-Quercetum ilicis typicum* plant association and *Myrtus communis* facies (Brullo et al. 1977) is dominated by *Quercus ilex* L. in the tree layer and shrubs such as *Arbutus unedo* L., *Erica arborea* L., *Pistacia lentiscus* L., *Phillyrea latifolia* L., and *Myrtus communis* L. The degradation stages of the association are characterized by garrigues with *Rubus ulmifolius* Schott. and *Euphorbia dendroides* L. (Brullo et al 1977).

The three myxomycete species collected — identified as *Craterium aureonucleatum* (*Physaraceae*), *Perichaena quadrata* (*Trichiaceae*), and *Physarum mutabile* (*Physaraceae*) — are new records for Italy.

Materials & methods

The investigation was carried out weekly on Pantelleria from October 2007 to May 2009 in Mediterranean maquis and garrigues ecosystems. The collected samples were identified by observing sporocysts in a water solution under Leica DMLB microscope.

Nomenclature of the recorded taxa follows MycoBank (<http://www.mycobank.org/>). The macro- and microscopic features of peridium, stipe (if present), hypothallus, capillitium, columella and/or pseudocolumella, and spores were observed. The habitat type was noted for each recorded species. Voucher specimens were deposited in Herbarium Mediterraneum Panormitanum, Palermo (PAL).

Species recorded

Craterium aureonucleatum Nann.-Bremek.

FIG. 1

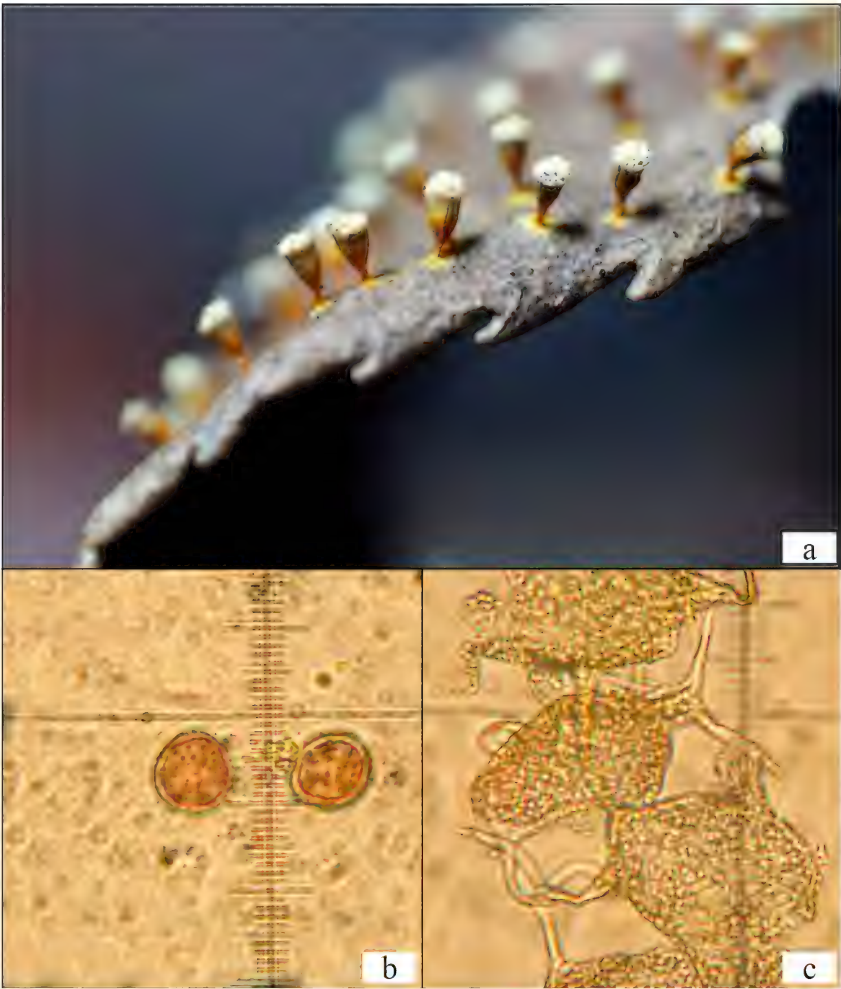


FIG. 1: *Craterium aureonucleatum*. [PAL 001(Myz)]
a. Sporocysts. b. Spores. c. A net with yellow nodes in the capillitium.

Sporocysts with a short stem, grouped, sub-conical, 0.3–0.5 mm in diameter and height, orange-dull brown. Hypothallus like a wide membranous disk, ridged, orange, stem not exceeding 0.2 mm in height. Peridium with two appressed layers, translucent. The outer layer of peridium is rather uniform while the inner layer is membranous. The two layers are orange at the base, colorless or yellow in the upper part. The dehiscence of the peridial apex is circular, forming a cup-shaped structure. The capillitium consists of a net with small meshes, pale yellow with numerous cells, often branched, calcareous yellow knots, pseudocolumella orange-yellow. Spore print dark brown; spores dark brown-lilac in transmitted light, 9–10 μm diam., covered by light tiny warts.

SPECIMEN EXAMINED: ITALY, SICILY, TRAPANI PROVINCE, **Pantelleria**, Cuddia di Mida and Montagna Grande, on litter of *Quercus ilex* L. and *Erica arborea* L. 10 Nov 2008, coll. R. Compagno [PAL 001 (Myz)].

COMMENTS – The sporocyst morphology in our collected specimens differs slightly from the original description of Ing (1999), where sporocysts are described as sub-globose, not sub-conical. This difference may be due to ecological conditions and infraspecific variability.

Craterium aureonucleatum has been recorded from Canary Islands, Germany, Netherlands, and Scotland (Beltrán & al. 2004, Ing 1999, Nannenga-Bremekamp 1991).

Perichaena quadrata T. Macbr.

FIG. 2



FIG. 2: *Perichaena quadrata* [PAL 002(Myz)]. a. Sporocysts. b. Spores and capillitium.

Sporocysts rarely single, usually appressed with polyhedral shape, discoid, sometimes lengthened, purple-brown or entirely black, with exudates of calcium oxalate, 0.1–0.5 mm in diameter. Hypothallus extended under the sporocysts, dark brown. Peridium double, initially adherent, the other layer thick and opaque, brownish with inclusion of the inner membrane and translucent, pale yellow, smooth. The capillitium consists of thin tubules, usually elastic and abundant, sometimes with rings that segment the tubules. Spore print yellow; spores yellow in transmitted light, 9–12 μm diam., lightly punctate.

SPECIMEN EXAMINED: ITALY, TRAPANI PROVINCE, **Pantelleria**, Bugeber, on dead stems of *Rubus ulmifolius* Schott, 15 Feb 2009, coll. R. Compagno [PAL 002 (Myz)].

COMMENTS – *Perichaena quadrata* has also been recorded from Africa, Asia, Australasia, Europe (including France and Spain), and North America (Lado 1994, The Eumycetozoon Project – <http://slimemold.uark.edu/index.htm>).

Physarum mutabile (Rostaf.) G. Lister

FIG. 3



FIG. 3: *Physarum mutabile* [PAL 003(My)]. a. Sporocysts. b. Spores.

Sporocysts with a short stipe; sub-globose; 0.3–0.6 mm wide and 2 mm long; white or pale grey. Hypothallus membranous, colorless, pale yellow or pale brown, sometimes with exudates. If present, the stipe is short, yellow to ochre,

usually covered by exudates. Peridium single, thin, colorless, smooth or rarely with calcium carbonate flakes, upper crack irregular. Capillitium persistent, elastic, with small meshes and white nodules. Pseudocolumella located in the center of sporocyst or at the base of plasmodiocarp. Spore print black; spores purple-brown in transmitted light, 7–10 µm diam., densely spinulose with spines sometimes grouped.

SPECIMEN EXAMINED: ITALY. SICILY, TRAPANI PROVINCE, **Pantelleria**, Montagna Grande, on litter of *Q. ilex* L. and *E. arborea* L., 18 Oct 2007, coll. R. Compagno [PAL 003 (Myz)].

COMMENTS – *P. mutabile* has been recorded from Africa, Asia, Europe, North America and South America (Emoto 1977, Ing 199, Lado 1994, Nannenga-Bremekamp 1991, The Eumycetozoa Project – <http://slimemold.uark.edu/index.htm>).

Conclusions

The new records of *C. aureonucleatum*, *P. quadrata* and *P. mutabile* extend their known European distribution into Italy and add valuable information regarding substrata.

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MYCOTAXON

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***Tuber microsphaerosporum* and *Paradoxa sinensis* spp. nov.**LI FAN^{1*}, JIN-ZHONG CAO² & YU LI²¹ College of Life Science, Capital Normal University,
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ABSTRACT — Two new truffle species from China are described and illustrated. *Tuber microsphaerosporum* is recognized by its small ascospores and 1–7-spored asci. *Paradoxa sinensis* is separated from the European *P. monospora* by its pale color and larger ascospores.

KEY WORDS — *Ascomycota*, mushroom market, *Tuberaceae*

Introduction

Knowledge about truffles in China has become richer since the first publication on *Tuber* in China by Liu (Liu 1985). Of the many species found, a high percentage has proved endemic (Wang 1988; Chen et al. 2005, 2007; Fan et al. 2011, 2012a, b). Recently we obtained two truffle-like fungi from a mushroom market in Kunming that we recognized as two new truffle species, a *Tuber* and a *Paradoxa*, described and illustrated here.

Materials & methods

The fresh fruiting bodies were collected from a local mushroom market in Kunming, China. Macro- and microscopic characters were described from fresh specimens. Sections were cut by razor-blade and mounted in 3% KOH for observation or stained in Melzer's reagent, rinsed, and mounted in polyvinyl lactic glycerol for permanent slides for archiving to be kept with dried specimens. For scanning electron microscopy (SEM), ascospores were scraped from the dried gleba onto doubled-sided tape, which was mounted directly on an SEM stub, coated with gold-palladium, and examined and photographed with a Hitachi S-4800 SEM. The specimens are deposited in BJTC (Herbarium Biology Department, Capital Normal University).

Taxonomy***Tuber microsphaerosporum* L. Fan & Yu Li, sp. nov.**

MYCOBANK MB 564356

FIG. 1

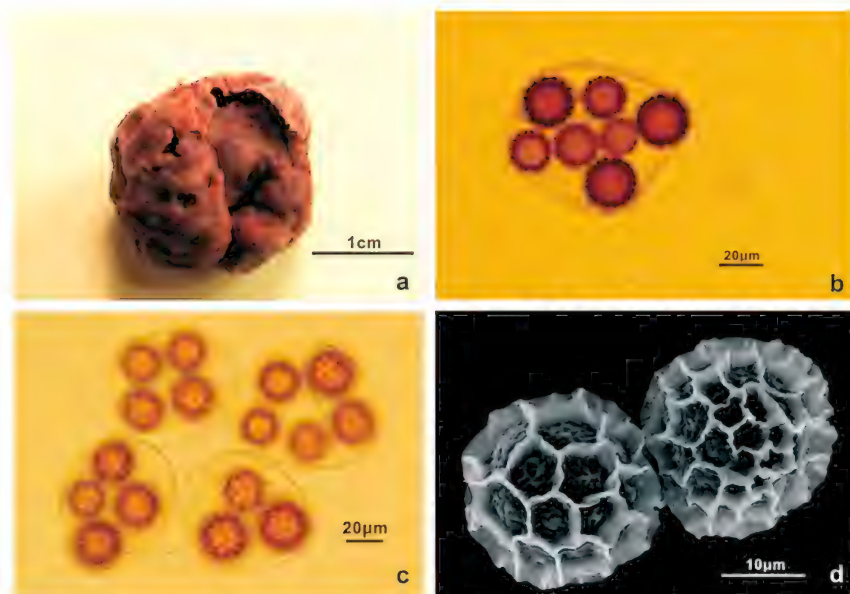


FIG. 1. *Tuber microsphaerosporum* (BJTC FAN152, holotype)
a. Ascoma. b–c. Asci and ascospores observed under light microscope.
d. Ascospores observed under SEM.

Differs from other *Tuber* species with globose ascospores by its smaller ascospores, up to 7 per ascus.

TYPE: China. Yunnan Province, Kunming, from a mushroom market. 20 Dec. 2010, Jin-Zhong Cao 115 (Holotype, BJTC FAN152).

ETYMOLOGY: *microsphaerosporum* (Lat.), referring to the small globose ascospores.

ASCOMA 2 cm in diam., subglobose, soft or slightly firm, solid, surface smooth, poorly pubescent to nearly glabrous, yellowish white to light yellow-brown at maturity. Odor slight, not pungent. PERIDIUM 250–350 μm thick, two layers; outer layer 100–150 μm thick, pseudoparenchymatous, composed of subangular or subglobose cells mostly 12.5–30 μm in diam., with thin or slightly thickened walls, pale yellowish, intermixed with larger swollen cells of 70–80 $\times$ 40–50 μm with thickened walls 2.5–3 μm in diam.; inner layer 150–200 μm thick, composed of intricately interwoven hyphae, thin-walled, branching, hyaline, septate, 3–5 μm in diam. The outermost cells giving rise a few hyphae-like hairs, the hairs 20–50 $\times$ 2.5–3 μm , septate, thin-walled, light brown. GLEBA light brown at maturity, marbled with large, rarely branching and white veins continuous with inner peridium. ASCI globose, subglobose or broadly ellipsoid, hyaline, thin-walled, 67.5–105 $\times$ 60–70 μm , mostly sessile, sometimes with a short stalk, 1–7-spored, mostly 3–6-spored. ASCOSPORES

globose, brown at maturity, 17.5–27.5 μm in 2–7-spored asci and 32.5–37.5 μm in 1-spored asci excluding ornamentation; ornamentation reticulate, meshes regular or irregular, 3–6 sided, 2.5–3 μm tall, the meshes generally 3–8 across the spore width.

COMMENTS — *Tuber microsphaerosporum* differs from the other *Tuber* species with globose ascospores mainly by its smaller ascospores and the asci containing 1–7 ascospores; moreover, its peridium structure with large swollen cells is also diagnostic for this new species.

The North American *Tuber californicum* Harkn. and *T. sphaerosporum* Gilkey and European *T. oligospermum* (Tul. & C. Tul.) Trappe and *T. borchii* var. *sphaerosperma* Malençon also produce perfectly globose ascospores, but all also produce 1–4- or 1–3-spored asci. *Tuber sphaerosporum* is further distinguished by large reticulum meshes numbering 3–4 across the spore diameter while *T. californicum* has a dark-colored mature gleba and *T. oligospermum* has a one-layer peridium.

Although we found no 8-spored asci in our holotype, 7-spored asci are not uncommon; it is possible that 8-spored asci may occur in the new species.

***Paradoxa sinensis* L. Fan & J.Z. Cao, sp. nov.**

FIG. 2

MYCOBANK MB 564357

Differs from *Paradoxa monospora* by its yellowish to yellow-brown ascomata and larger ascospores.

TYPE: China. Yunnan Province, Kunming, from the local mushroom market. 20 Dec. 2010, Jin-Zhong Cao 113 (Holotype, BJTC FAN150).

ETYMOLOGY: *sinensis* (Lat.), referring to the presumed host country.

ASCOMA 2.5 cm in diam., subglobose, firm, solid, surface smooth, glabrous, yellowish to yellow-brown at maturity. Odor slight, not pungent. PERIDIUM 200–250 μm thick, one layer, prosenchymatous, composed of intricately



FIG. 2. *Paradoxa sinensis* (BJTC FAN150, holotype)
a. Asci and ascospores observed under light microscope. b. Ascoma.

interwoven hyphae, branching, septate, the hyphae are light yellow-brown, slightly thick-walled and 5–7.5(–10) μm broad towards the outer side of peridium, and hyaline, thin-walled and 5–7.5 μm broad towards the inner side. GLEBA blackish at maturity, marbled with large, rarely branching and greyish or grey-brown veins continuous with the peridium. ASCI 1-spored, rarely 2-spored, globose or subglobose for 1-spored and long-ellipsoid for 2-spored, 77.5–92.5 $\times$ 80–85 μm for 1-spored and 120 $\times$ 80 μm for 2-spored, hyaline, thick-walled, 5–7.5 μm thick, sessile or with a short tail. ASCOSPORES globose, red-brown to dark red-brown at maturity, (55–)62.5–72.5 μm in diam. excluding ornamentation, ornamentation reticulate, irregular, meshes varying greatly in shape and size, 5–20 μm in diam., 1 μm high, averagely 6–10 across the spore width.

COMMENTS — *Paradoxa sinensis* closely resembles the European *P. monospora* Mattir., but Ceruti (1960) describes the ascomata of *P. monospora* as black brown and the ascospores as somewhat smaller (50–60 μm in diam.). The third *Paradoxa* species, *P. gigantospora* (Y. Wang & Z.P. Li) Y. Wang (Wang & Hu 2008), was originally described from China by Wang & Li (1991, as *Tuber gigantosporum*) and is diagnosed by its large elliptic ascospores ($\leq 100 \mu\text{m}$).

Paradoxa, originally described from Italy in 1935, was monotypic until Wang & Hu (2008) transferred *T. gigantospora* to it. *Paradoxa sinensis* represents the third species in the genus, which differs from *Tuber* mainly by its single-spored asci. Vizzini (2008), who treated *Paradoxa* as a synonym of *Tuber*, transferred *P. monospora* to *Tuber* as *T. monosporum*. Kinoshita et al. (2011) seemingly accepted this classification when they listed *Paradoxa gigantospora* as a synonym of *Tuber gigantosporum*. However, in all three *Paradoxa* species, the dominant character of one larger ascospore per ascus is always connected with peculiar reticulum on the spore surface, which is highly irregular and greatly lower, suggesting a taxonomic difference between *Paradoxa* and most typical *Tuber* species. More research is needed to elucidate whether the two genera are independent or conspecific. We prefer to treat *Paradoxa* and *Tuber* as independent genera for the time being.

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***Entomophthoromycota*: a new phylum and reclassification for entomophthoroid fungi**

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ABSTRACT — One result of the recent phylogenetically based rejection of the phylum *Zygomycota* was the description of the subphylum *Entomophthoromycotina* (not assigned to any phylum) for fungi traditionally treated in the order *Entomophthorales*. More extensive gene-based analyses of these fungi suggest that they represent a monophyletic lineage distinct from all other fungi that deserves now to be recognized at the level of a new fungal phylum. These molecular data and further analyses of more traditional taxonomic criteria lead to this reclassification that still treats these fungi in six families but recognizes the new classes *Basidiobolomycetes*, *Neozygitomycetes*, and *Entomophthoromycetes* as well as the new order *Neozygiales*. *Ballocephala* and *Zygnemomyces* are excluded from *Entomophthorales* (*Meristacaceae*) and should be reclassified among the *Kickxellomycotina*.

KEY WORDS — *Zygomycetes*, sexuality, homothallism

Introduction

The reclassification of fungi by Hibbett et al. (2007) as the complement to a kaleidoscopic phylogenetic study all major fungal groups (James et al. 2006) validated the long-accepted sense that *Zygomycota* was polyphyletic, and recognized five taxa to replace this phylum: The phylum *Glomeromycota* accommodates arbuscular mycorrhizal fungi, and all other zygomycetes were distributed among subphyla *Entomophthoromycotina*, *Kickxellomycotina*, *Mucoromycotina*, and *Zoopagomycotina* without assignment to any phylum. It was assumed that subsequent research would determine whether any of these subphyla should be regrouped as part of an effort that would necessarily result in the recognition of one to four new phyla for these fungi.

The major characters traditionally used to classify the *Entomophthorales* have been thoroughly reviewed (summarized in Humber 1975, 1981, 1982,

1984) and were applied to six families in the last major reclassification of *Entomophthorales* (Humber 1989); this taxonomy is widely accepted despite a few minor differences in the treatments of some entomopathogenic genera (Bałazy 1993; Keller 1987, 1991, 1997; Keller & Petrini 2005). Until now, however, there have not been phylogenetic studies on a sufficiently broad range of their genes and taxa to propose a more contemporary revision.

The fungi in *Entomophthoromycotina* pose a few mycological puzzles (discussed below) for refining their current classification: Both the ultrastructure of the mitosis-associated organelle and early phylogenetic studies suggested that *Basidiobolus* has affinities with chytrid fungi and might better be excluded from *Entomophthorales*. Further, significant gaps in the gene-based understandings of entomophthoroid fungi exist because many taxa are very rarely collected and/or resist growth in vitro. Among these understudied taxa, *Neozygites* and related species represent the largest and most important 'black boxes' for which needed data remain unavailable.

The gene sequences now conceded to have taxonomic value for many fungi (nuclear rDNA genes, translation-elongation factor, β -tubulin, etc.) have been used in studies of a few entomophthoraleans in a more diverse set of fungi (Nagahama et al. 1995, Jensen et al. 2001, White et al. 2006) and for narrower studies of entomophthoralean species or species complexes (Jensen & Eilenberg 2001, Nielsen et al. 2001). A markedly different molecular approach comparing amino acid sequences for proteins (including some of the same proteins whose DNA sequences are widely used) has placed *Entomophthorales* outside of the true fungi (Einax & Voigt 2004, Liu & Voigt 2010, Voigt & Kirk 2011). While some skepticism about the meaning of such results based on amino acid sequences must be maintained, these findings do demonstrate the existence of distinct differences between all other groups of zygomycete fungi and *Entomophthoromycotina*. The amino-acid sequence-based 'exclusion' of *Entomophthorales* from the fungi echoes the hypotheses about placing *Basidiobolus* with water molds but such 'anomalous' conclusions also suggest that molecular analyses based on highly limited inputs can yield results that have little sensibility from the broader perspective of the overall biology of the organisms analyzed.

A series of phylogenetic studies on entomophthoraleans (being prepared by A. Gryganskyi, R. Vilgalys, R. Humber, and other authors) incorporated more genes and a much broader range of entomophthoroid taxa than any earlier studies. These new analyses confirm the finding by James et al. (2006) that entomophthoroid fungi are a monophyletic group and that this group does include *Basidiobolus* and *Basidiobolaceae*. A reasonable integration of all results of traditional and phylogenetic analyses of entomophthoroid fungi suggests that they are distinct from all other fungi (including those in the other

unaffiliated zygomycete subphyla; Hibbett et al. 2007) and may occupy the most basal position among all non-flagellate fungi. The best-supported, most appropriate conclusion about the status of these fungi is, therefore, to recognize them as a new phylum in kingdom *Fungi*.

Materials & methods

Cultures and specimens used for the analyses discussed here are primarily from the USDA-ARS Collection of Entomopathogenic Fungal Cultures (ARSEF; <http://www.ars.usda.gov/Main/docs.htm?docid=12125>) and its associated herbarium. Unpublished molecular results of analyses of various genes are based on sequences of cultures obtained from ARSEF or isolated from nature and, in most cases, subsequently deposited in ARSEF. Other results involving reports on fungi and analyses using other isolates and specimens were completed at the Zoology Section of the Department of Agriculture and Ecology, University of Copenhagen, or at Agroscope FAL Reckenholz Eidgenössische Forschungsanstalt für Agrarökologie und Landbau (Zürich).

Major taxonomic issues affecting this reclassification

'Linkage' of *Basidiobolus* with flagellate fungi

The nuclei of entomophthoralean fungi and the details of their mitoses present a comparatively richer number and variety of characters than in most other fungal groups, and these nuclear characters are taxonomically informative, especially above the generic rank (Humber 1975, 1981, 1982, 1984, 1989). Mitosis by the huge nuclei of *Basidiobolus* species is sufficiently unusual to have been studied repeatedly (Olive 1907, Robinow 1963, Sun & Bowen 1972, Tanaka 1978). Among other surprises, the location of the mitosis-associated organelle in this genus is not fixed at the spindle poles and can even occur in the plane of the metaphase plate (Sun & Bowen 1972). The real controversies about this mitosis arose, however, when this organelle proved to be a short cylinder with a ring of 11–12 singlet microtubules (McKerracher & Heath 1985), and comparisons between this structure and centrioles were used to question the phylogenetic placement of this fungus; *Basidiobolus* remains the ONLY organism producing no flagellum in its life history for which microtubules are proven to be present in a mitosis-associated organelle. Nonetheless, any hypothesis that this organelle in *Basidiobolus* (but whose existence, location and ultrastructure remain unconfirmed from other fungi in its family) is linked with or derived from centrioles seems neither credible nor responsible: The 9×3 microtubular arrangement in centrioles and kinetosomes is invariant among ALL phyla of eukaryotes having flagella; conversely, no organelle with microtubules arranged like those in *Basidiobolus* is known from any other organism.

Later findings of gene sequence similarities between *Basidiobolus* and several chytrid and blastocladian water molds (Nagahama et al. 1995, Jensen et al. 1998, Tanabe et al. 2000, White et al. 2006) have also been used to suggest

that *Basidiobolus* might not belong in *Entomophthorales*. However, other studies allied *Basidiobolus* with kickxelloid fungi from *Harpellales* (Keeling 2003, Tanabe et al. 2004) and one placed *Conidiobolus coronatus* (whose inclusion in *Entomophthorales* was never disputed) on a branch with the blastocladialean genus *Allomyces* (Tanabe et al 2005). These divergent findings underscore the peril of suggesting phylogenetic relationships among major fungal groups after comparing very limited sequence data and very sparse samplings of taxa within large and inherently diverse groups of fungi.

The recent survey of phylogenetic relationships within kingdom *Fungi* (James et al. 2006) supported the continued placement of *Basidiobolus* in *Entomophthorales*. Regardless of molecular or ultrastructural rationales to the contrary, hypotheses that *Basidiobolus* is not entomophthoroid are nonsensical if one considers the overall biology of these fungi. *Basidiobolus* and its relatives share many novel features in common with other entomophthoroid taxa despite the scant few pieces of data suggesting otherwise. It is necessary to bring a broader perspective to the uncertainties about *Basidiobolus*: Despite any and all evidence to the contrary, if this genus does not belong among the entomophthoroid fungi, then JUST WHERE AMONG FLAGELLATE (OR ANY OTHER) FUNGI SHOULD IT BE CLASSIFIED? The lack of any comparably well-supported answers for this question should quash any residual doubts about where *Basidiobolus* belongs.

***Neozygitaceae*: a special 'problem' in data gathering**

The status of taxa in *Neozygitaceae* also presents a (temporary) problem for reclassifying the *Entomophthorales*. Very few cultures of *Neozygites* species are available in vitro, and, sadly, all current cultures of *Neozygites* are of mite pathogens with rough-walled, nearly globose zygospores. No gene-based data are available for *N. fresenii* [= *N. aphidis*, the type species] or other hemipteran pathogens that form ovoid, smooth-walled resting spores. A further taxonomic frustration is that these morphological and host differences suggest that *Neozygites* may eventually be split into two or more genera, but molecular data will be required to support such a decision. DNA-based evaluations of fungi from *Neozygitaceae* is encumbered by difficulties experienced in multiple laboratories to obtain clean DNA useful for amplifying and sequencing the genes needed to determine their phylogenetic relationships. While the recognition of *Neozygitomycetes* as a new class without supporting gene-based evidence may be dismissed by some as premature, such a treatment is the most reasonable for these fungi at this time, based on their known organismal biology (that integrates and represents a vastly larger proportion of the genome than those few genes now so widely treated as sufficient to complete high-level, phylogenetically sound reclassifications of virtually all other fungal groups).

Distinct differences between neozygoid fungi and either basidioboloid or entomophthoroid taxa supports the description of three classes in this new phylum: As in basidioboloid fungi, neozygoid fungi exert strong control in vegetative cells over nuclear number (usually four in *Neozygites*), have a central mitotic metaphase plate (Butt & Humber 1989), and, perhaps most significantly, a round of mitosis in gametangial cells precedes conjugation and zygosporogenesis while only one nucleus from each gametangium enters each zygospore (Keller 1997). As in entomophthoroid fungi, all neozygoid taxa are obligate pathogens of insects or mites, and the nuclear membrane remains intact throughout mitosis. On the other hand, the chromosomes of neozygoid fungi differ from basidioboloid and entomophthoroid fungi in being vermiform and of moderate size, condensing during mitosis but uncoiled (euchromatic) during interphase. *Neozygites* mitoses (Butt & Humber 1989) resemble those in animal or plant cells more closely than those in any other entomophthoroid fungi.

The presence of many novel characteristics shared among all of the fungi traditionally classified in order *Entomophthorales* underscores the need to keep these fungi together in a phylogenetically supported, coherent group and to pursue further studies to obtain more vital data about the genes, development, pathobiology, and other aspects for a better understanding of these fungi that can be very important naturally occurring biological control agents. Because these fungi occupy a very ancient position of the fungal tree of life it is also important to note that a better understanding should help to understand more about the enigmatic transition of fungi (as also occurred with plants and animals) from waterborne to terrestrial life forms.

Taxonomy

Entomophthoromycota Humber, phyl. nov.

[TABLE 1]

MYCOBANK MB 564375

VEGETATIVE GROWTH AS hyphae, hyphal bodies, or yeast-like; cells broad, walled or protoplasmic. CONIDIOPHORES simple or digitate, each branch forming one conidiogenous cell and one conidium. PRIMARY SPORES conidia, uni- to multinucleate, usually forcibly discharged; usually forming one or more types of SECONDARY CONIDIA. RESTING SPORES homothallic zygospores or azygospores. HABIT mostly as arthropod pathogens, but some saprobes or specialized phytopathogens.

TYPE GENUS: *Entomophthora* Fresen. 1856.

CONIDIOPHORES rise from mycelium or from body of host, usually with positive phototropic orientation, simple or apically (digitately) branched, with single conidiogenous cell on each branch giving rise to a single conidium or simple erect conidiophore becomes septate and each cell forms a single conidium. PRIMARY CONIDIA (not sporangia) with wall layers continuous with those on

TABLE 1. Proposed new classification for *Entomophthoromycota*.

New taxa described here are listed in *boldface italics*.

PHYLUM *Entomophthoromycota* Humber, phyl. nov.

CLASS *Basidiobolomycetes* Humber, cl. nov.

ORDER *Basidiobolales* Caval.-Sm., Biol. Rev. 73: 246. 1998.

FAMILY *Basidiobolaceae* Claussen, Syllab. Pflanzenfam., Edn 9 & 10: 45. 1924.

Basidiobolus Eidam, Beitr. Biol. Pflanz. 4: 194. 1886.

Other new, undescribed genera (R.A. Humber, B.

Huang & K. Hodge, unpublished).

CLASS *Neozygitomycetes* Humber, cl. nov.

ORDER *Neozygiales* Humber, ord. nov.

FAMILY *Neozygitaceae* Ben-Ze'ev, R.G. Kenneth &

Uziel, Mycotaxon 28: 321. 1987.

Apterivorax S. Keller, Sydowia 57: 47. 2005.

Neozygites Witlaczil, Arch. Mikr. Anat. 24: 601. 1885.

Thaxterosporium Ben-Ze'ev & R.G. Kenneth, Mycotaxon 28: 323. 1987.

CLASS *Entomophthoromycetes* Humber, cl. nov.

ORDER *Entomophthorales* G. Winter, Rabenh. Krypt.-Fl., Edn 2, 1(1): 74. 1880.

FAMILY *Ancylistaceae* J. Schröt., Nat. Pflanzenfam. 1(1): 92. 1893.

Ancylistes Pfitzer, Monatsb. Königl. Preuss. Akad. Wiss. Berlin: 396. 1872.

Conidiobolus Bref., Untersuch. Gesammtgeb. Mykol. 6: 37. 1884.

Macrobotophthora Reukauf, Centrabl. Bakt., Abt 1, 63: 390. 1912.

FAMILY *Completoriaceae* Humber, Mycotaxon 34: 453. 1989.

Completoria Lohde, Tagebl. Versamml. Deutsch. Naturf. Aertze 47: 206. 1874.

FAMILY *Entomophthoraceae* Nowak., Bot. Ztg. 35: 35. 1877.

SUBFAMILY *Entomophthoroideae* S. Keller, Sydowia 57: 28. 2005.

Batkoa Humber, Mycotaxon 34: 446. 1989.

Entomophaga A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 325. 1964.

Entomophthora Fresen., Bot. Ztg. 14: 883. 1856.

Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.

Massospora Peck, Rep. New York State Mus. 31: 44. 1879.

SUBFAMILY *Erynioideae* S. Keller, Sydowia 57: 33. 2005.

Erynia (Nowak. ex A. Batko) Remaud. &

Hennebert, Mycotaxon 11: 333. 1980.

Eryniopsis Humber, Mycotaxon 21: 258. 1984, pro parte.

Furia (Batko) Humber, Mycotaxon 34: 450. 1989.

Orthomyces Steinkraus, Humber & J.B. Oliv., J. Invertebr. Pathol. 72: 5. 1998.

Pandora Humber, Mycotaxon 34: 451. 1989.

Strongwellsea A. Batko & Weiser, J. Invertebr. Pathol. 7: 463. 1965.

Zoophthora A. Batko, Bull. Polon. Acad. Sci. Sér. Biol. Sci. 12: 323. 1964.

FAMILY *Meristacraceae* Humber, Mycotaxon 34: 456. 1989.

Meristacrum Drechsler, J. Wash. Acad. Sci. 30: 250. 1940.

Tabanomyces Couch, RV Andrejeva, Laird & Nolan,

Proc. Natl. Acad. Sci. USA 76: 2302. 1979.

conidiogenous cells, inner wall layer invaginating to form two-layered septum between conidium and conidiogenous cell; almost always forcibly discharged (several possible mechanisms are known). SECONDARY CONIDIA formed by most taxa: if forcibly discharged from short secondary conidiophore then usually similar in shape to primary conidium; if passively dispersed from long, thin (capillary) secondary conidiophore then usually distinctly differing in morphology from primary conidium. RESTING SPORES (when mature) with thick, distinctly 2-layered walls, colored or hyaline, outer layer surface smooth or variously decorated; formed as zygosporangia (after gametangial conjugation) or azygosporangia (with no conjugation) either in the axis of the parental cells or budded off laterally; nuclear number in mature spores varies from 2 (from initiation or progressively reducing to 2) to multiple; germinating directly by forming germ conidiophore and germ conidium (usually resembling a secondary spore type) or indirectly by forming a small germ mycelium and then germ conidia (usually like primary conidia). HABITS: saprobes in soil or litter, primary pathogens of arthropods (insect, mites, spiders) or other soil invertebrates (nematodes, tardigrades), or highly specific phytopathogens (e.g., of desmid algae or fern gametophytes). ARTHROPOD PATHOGENS may form specialized organs: RHIZOIDS with or without differentiated holdfasts may anchor host to substrate, and CYSTIDIA may perforate host cuticle and facilitate emergence of conidiophores.

Primary and secondary conidia are the major spore forms in this phylum and constitute the primary basis for the taxonomy of these fungi. The resting spores are formed much less commonly than are conidia. The majority of species are pathogens of arthropods although pathogens of other soil invertebrates (nematodes and tardigrades) or of plants (desmid algae or fern gametophytes) are rare. The primary habit (especially in *Basidiobolaceae* and *Ancylistaceae*) may be in soil and plant detritus, but some species in these groups are best known as colonists of amphibian and reptile guts (*Basidiobolus*) or as facultative or obligate entomopathogens (*Conidiobolus*).

Any continued use of subphylum *Entomophthoromycotina* Humber (Hibbett et al. 2007: 517) is now superfluous until any future decision divides *Entomophthoromycota* into subphyla. This reclassification does not take up the phylum *Basidiobolomycota* Doweld (2001: LXXVII) because Doweld's name was proposed as part of a general reclassification of all fungi that does not agree with current understandings of fungal biology and relationships and, as circumscribed, *Basidiobolomycota* and the class *Basidiobolomycetes* Doweld (2001: LXXVII) used fragmentary knowledge of characters that may not apply to all taxa intended to be included while failing to account in any way for most taxa specifically included in this circumscription of phylum *Entomophthoromycota*; also see discussion below for class *Basidiobolomycetes*.

***Basidiobolomycetes* Humber, cl. nov.**

MYCOBANK MB 564376

Differs from *Entomophthoromycetes* and *Neozygitomycetes* by unusually large nuclei (often $\geq 10\ \mu\text{m}$ long) with a large central nucleolus that is the major feature of uninucleate cells. Mitoses involve barrel-shaped spindles, mitotic organelles incorporating microtubules (but not centrioles) but not always located at the spindle poles, and the nuclear content isolated from the cytoplasm by a layer of nuclear and cytoplasmic membrane fragments.

TYPE GENUS: *Basidiobolus* Eidam 1886.

VEGETATIVE CELLS uninucleate, as regularly septate mycelium or yeast-like cells cleaved from contents of a parental cells (e.g., so-called 'Darmform' growth). MITOSIS begins with fragmentation of nuclear membrane and aggregation of these and other membranes around a nuclear zone; chromosomes numerous, tiny, condensed and aligned on central metaphase plate (usually embedded inside the nucleolus) in association with a barrel-shaped spindle, chromosomes uncoil during interphase. CONIDIOGENOUS CELL (CONIDIOPHORE) simple but with bulbous apical swelling immediately below developing conidium. CONIDIA uninucleate, globose, with small conical basal papilla (projecting into spore body but everting during discharge), unitunicate (wall layers not separable). CONIDIA DISCHARGE forcibly by rocket-like ejection when central circumscissile weakness of the subconidial swelling ruptures; the upper portion of the swelling discharges together with conidium but may detach during flight. SECONDARY CONIDIA (if formed) usually elongate, often curved, with or without an apical mucoid droplet, formed apically on an elongated capillary conidiophore, passively dispersed. RESTING SPORES (RS) usually zygospores, formed homothallically in axis of parental cells; gametangial nuclei undergo mitosis before conjugation but only one nucleus from each cell enters the zygospore. MATURE ZYGOSPORES have thick, bi-layered walls; RS GERMINATE by direct formation of germ conidium (usually a secondary conidial type: elongate, passively dispersed from a capillary conidiophore).

The foremost diagnostic character for basidioboloid fungi is their huge nucleus (often $\geq 10\ \mu\text{m}$ in length) with a prominent central nucleolus that is the major feature of uninucleate cells (either as a broad, septate mycelium or cells cleaving internally in yeast-like growth mode). There is no staining of interphasic nuclei (nor, in any obvious manner, of mitotic chromosomes) in *Basidiobolomycetes* in aceto-orcein or other nuclear stains. The individual volumes of these nuclei may be many times greater than the entire cells of most ascomycete yeasts), and their mitoses are unusual for more than just the microtubular nucleus-associated organelle (McKerracher & Heath 1985): As mitosis begins, the nuclear envelope breaks down but the endoplasmic reticulum and other membranous cell components cluster around the nuclear zone so that the

spindle and chromosomes remain well isolated from the cytoplasm despite the fragmentation of the nuclear envelope; the corollary effect of this membrane organization is that mitotic nuclei 'disappear' when viewed with light microscopy (Robinow 1963).

Zygosporogenesis in *Basidiobolus* (Eidam 1886) is also very distinctive as short beak-like, lateral projections form at the septum between gametangial cells; gametangial nuclei move into the beaks, undergo mitosis, and the (uninucleate) beak cells are walled off before the septum dissolves and zygosporogenesis proceeds; remnants of these 'beaks' often remain visible on mature zygosporoes.

As noted in the discussion for the new phylum, two available names for this new class were not adopted: *Bolomycetes* Cav.-Smith (Cavalier-Smith 1998: 243) was based mainly on the microtubular mitotic organelle and 'beaked' zygosporoes in *Basidiobolus*. This mitotic organelle is not confirmed as present in all taxa in the *Basidiobolaceae* (including at least two still undescribed new genera), and the zygosporoes of some basidiobolaceous fungi are not 'beaked' as in *Basidiobolus*. *Basidiobolomycetes* Doweld (2001: LXXVII) was proposed as a nomen novum for *Bolomycetes* and cited Cavalier-Smith's description for this class; Doweld neither placed nor mentioned other entomophthoralean fungi in any rank in his general reclassification of fungi.

Neozygitomycetes Humber, cl. nov.

MYCOBANK MB 564377

Differs from *Basidiobolomycetes* and *Entomophthoromycetes* by vermiform, moderately sized chromosomes that condense during mitosis on a central metaphase plate but uncoil during interphase. Nuclear numbers in vegetative cells and conidia are low and apparently controlled at (3)-4-(5).

TYPE GENUS: *Neozygites* Witlaczil 1885.

VEGETATIVE CELLS are rod-like hyphal bodies, walled or protoplasmic, usually with 4 (3-5) nuclei, elongating until $\pm$ synchronous mitosis; daughter cells separate by splitting of septum. NUCLEAR NUMBER in all cell types strongly regulated; usually 4 (3-5) in vegetative cells and conidia, 2 in resting spores. MITOSES intranuclear, $\pm$ synchronous in any cell; nuclei fusoid at metaphase with central, fusoid spindle; no nucleus-associated mitotic organelle observed; chromosomes uncoil (euchromatic) during interphase. CONIDIOPHORES simple; forming apical conidiogenous cell and one conidium. PRIMARY CONIDIA subglobose to broadly ovoid, basal papilla short, comparatively flat; forcibly discharged to short distance by papillar eversion. SECONDARY CONIDIA usually form quickly after primary conidial discharge, most commonly form as capilliconidia (that are the primary infective units). RESTING SPORES bud from short conjugation bridge between rounded-up hyphal bodies (gametangia)

after preconjugal mitosis in contacting gametangia; zygospore receives one nucleus from each gametangium; only outer wall layer is melanized. Mature resting spores with two adjacent round fenestrae ('holes' through outer wall layer) and raised ridge of gametangial wall remnants between them.

Melanization of all spore types is a major feature of *Neozygitomycetes*. Primary and secondary conidia are pale, smoky gray; individual resting spores are much more strongly colored, and dark gray to black in mass.

***Neozygiales* Humber, ord. nov.**

MYCOBANK MB

ORDER having all characteristics of class *Neozygitomycetes*.

TYPE GENUS: *Neozygites* Wiltacil 1885.

This order has all characters of class *Neozygitomycetes* (which includes only a single order and family).

***Entomophthoromycetes* Humber, cl. nov.**

MYCOBANK MB 564381

Differs from *Basidiobolomycetes* by lack of uniformly uninucleate cells, nuclear morphology, details of mitoses, and modes of zygosporogenesis; and from *Neozygitomycetes* by cells not having uniformly small numbers of nuclei, details of mitoses, and lack of melanization of all spore types.

TYPE GENUS: *Entomophthora* Fresen. 1856.

VEGETATIVE GROWTH as coenocytic mycelium or rod-like to variably shaped hyphal bodies, walled or naturally protoplasmic; if wall-less, rod-like to highly variable in shape and amoeboid. CONIDIOPHORES simple or digitately branched, each branch with a single apical conidiogenous cell, or (in *Meristacraceae*) an unbranched erect, septate conidiophore forming one conidium per cell. Conidia unitunicate (wall layers not separating in liquid) or bitunicate (with separable outer wall layer); variously shaped, uni- to multinucleate, with basal papilla flat, conical or rounded; forcibly discharged by papillar eversion in most genera. SECONDARY CONIDIA more or less similar in shape to primary conidia and forcibly discharged if formed on short secondary conidiophore, or elongate and passively dispersed if formed on elongated capillary secondary conidiophore. NUCLEI (interphase) with small nucleolus, interphasic heterochromatin present in *Entomophthoraceae* but absent in all other families; mitoses intranuclear, with small lateral metaphase plate lateral; interphasic chromosomes are partly condensed (heterochromatic) and stain readily in *Entomophthoraceae* but euchromatic (uncoiled and nonstaining) in other families. RESTING SPORES globose to subglobose, formed as zygospores or azygospores. HABIT obligately pathogenic for invertebrates (*Entomophthoraceae*, *Meristacraceae*, some *Ancylistaceae*), saprobic (some *Ancylistaceae*), or phytopathogenic (*Completozia* [*Completoziaceae*] and *Ancylistes* [*Ancylistaceae*]).

This class includes all members of *Ancylistaceae*, *Entomophthoraceae*, *Completoriaceae*, and *Meristacraceae* but omits those entomophthoralean taxa reassigned here to *Basidiobolomycetes* or *Neozygitomycetes* (TABLE 1) or removed from *Entomophthoromycota* as noted below.

Genera incertae sedis:

Eryniopsis Humber, Mycotaxon 21: 258. 1984.

All species are in *Entomophthoraceae* but would appear to be a mix of taxa representing both subfamilies *Erynioideae* and *Entomophthoroideae*; the type species, *E. lampyridarum*, has morphological characters suggestive of both subfamilies and cannot be placed in either without molecular studies.

Tarichium Cohn, Beitr. Biol. Pflanzen 1: 69. 1870.

This form genus for species known only from resting spores apparently represents a mix of species attributable to *Neozygitaceae* (especially species pathogenic to mites) and *Entomophthoraceae*. No new species should be added to this genus; DNA-based studies and morphological re-evaluations should allow most species to be recognized as synonyms of other species or transferred to other genera in *Entomophthoraceae* and *Neozygitaceae*.

Taxa inadoptata vel excludenda:

Massosporoideae S. Keller, Sydowia 57: 44. 2005.

This subfamily (accommodating only the genus *Massospora*) seems not to be supported by phylogenetic evidence and is treated as a synonym of subfamily *Entomophthoroideae*.

Ballocephala Drechsler, Bull. Torrey Bot. Club 78: 199. 1951.

Zygnemomyces K. Miura, Rep. Tottori Mycol. Inst. 10: 520. 1973.

These two genera are excluded from *Meristacraceae* and reassigned to *Kickxellomycotina* Benny (Hibbett et al. 2007) based on the bifurcate septa with lenticular plugs in their vegetative hyphae (Saikawa 1989; Saikawa et al. 1997).

Discussion

The terms 'mitospore' and 'meiospore' are not used in characterizing taxa of *Entomophthoromycota*. They were originally adopted to describe ascomycete and basidiomycete spores, and are not applicable to entomophthoroid fungi because the reproductive products and life histories of entomophthoroid fungi are not strictly comparable with those of the *Dikarya*: The thin-walled primary conidia (the basis for entomophthoroid taxonomy) are produced by the vegetative cells of these fungi, usually forcibly discharged, and usually able to produce one or more subsequent forms of secondary conidium if conidia do not germinate by producing a germ tube. Entomophthoroid resting spores may be conventionally sexual in nature (zygospores in which it may be ASSUMED, although not yet proven, that karyogamy and meiotic

divisions occur) in *Basidiobolomycetes* and *Neozygitomycetes*; for taxa in the *Entomophthoromycetes*, and especially those in *Entomophthoraceae*, it was noted that the MORPHOLOGICAL events of sexuality (the presence or absence of gametangial conjugations define zygo- and azygosporeogenesis, respectively) and the GENETIC events of sexuality (karyogamy and meiosis, that presumably happen during resting spore germination) may be completely independent processes (Humber 1981, McCabe et al. 1984). Entomophthoroid fungi may be the only fungi in which the morphological and genetic definitions of sexuality (or their absences) are present in all possible permutations and without the routine linkage between the morphological and genetic events of sexuality that is taken for granted in virtually all other types of organisms.

No zygomycetous or flagellate fungi produce spores that can accurately be referred to as meiospores in the sense of basidiospores and ascospores. The proven or presumably 'sexual' spores of fungi below the subkingdom *Dikarya* are thick-walled, environmentally resistant spores (zygospores, azygospores, resistant sporangia, etc.) that go through a quiescent dormancy before germinating to undergo a type of sporulation that is neither functionally nor developmentally comparable to the basidiospores and ascospores that are the direct and obligatory developmental products of the cells in which karyogamy and meiosis occur.

To obtain clean DNA and good sequence data from entomophthoroid fungi may be more difficult than for many, much more extensively studied fungal groups. Part of this difficulty might involve the physical organization of the genome in these fungi that might lead to overlapping but divergent sequences for some 'needed' genes. Chromosomal counts for *Basidiobolus* (which was long treated as including only one species, *B. ranarum*) have ranged from about 60 (Olive 1907) to several hundred (Sun & Bowen 1972) based on kinetochore counts in serial sections for transmission electron microscopy. These high numbers suggest that polyploidization events may have occurred repeatedly in *Basidiobolus*. This possibility seems to be verified by genetic studies showing multiple, genetically distinct allelic forms in *B. ranarum* for elongation-translation factor genes that usually occur in single copies in the genome (Henk & Fisher 2012). The few chromosome counts for entomophthoraceous entomopathogens also suggest a tendency to towards polyploidy: While the nature of chromosomes and mitoses may not facilitate chromosomal counts in *Entomophthoraceae* (Olive 1906, Humber 1975), the few published numbers in various taxa are 8, 12 (or more), 16, and 32 (see Humber 1982). No genetic studies like those of Henk & Fisher (2012) are available for *Entomophthoraceae* but no genetic studies of this family with techniques ranging from allozyme polymorphisms (Hajek et al. 1990; B. May, personal communication) to the latest gene sequencing efforts suggest that these fungi simultaneously harbor

multiple allelic variants at single loci; there is no indication that vegetative nuclei of these fungi are either diploid or include heterologous sets of chromosomes.

The interpretation of such seemingly numerous chromosomes in some taxa in *Entomophthoromycota* becomes more problematic in the absence of evidence suggesting that any putatively sexual reproduction in this phylum is heterothallic rather than strictly homothallic. No data support the invocation of heterothallic sexuality (even if outbreeding events were extremely rare) to explain Henk & Fisher's (2012) conclusions about the *Basidiobolus* genome. Except for gametangial fusions during zygosporogenesis, no cellular fusions (even between the naturally protoplastic vegetative cells of some of the pathogenic taxa) are known from cultures or natural collections of any entomophthoroid fungus; such fastidious behavior by these fungi precludes consideration of heterokaryosis and parasexuality as a mechanism to increase or to sustain gene flow among taxa in *Entomophthoromycota*.

It is important to note again that the *Entomophthorales* as traditionally recognized (Humber 1989) is the same group reclassified here except for the removal of *Ballocephala* and *Zygnemomyces* to *Kickxellomycotina* based on the bifurcate, plugged septa in their vegetative hyphae (Saikawa 1989, Saikawa et al. 1997). Despite earlier doubts about retaining *Basidiobolus* in *Entomophthorales*, molecular studies of more genes and a broader spectrum of entomophthoraleans (A. Gryganskyi, R. Vilgalys, and R. Humber, unpublished) confirm that this order, as historically treated, is monophyletic. These fungi exemplify yet another major group for which the traditional, pre-molecular classification has been fundamentally confirmed (although amplified and adjusted) rather than overturned by phylogenetic analyses. Phylogenetic techniques must not be allowed to override or to supplant the existing knowledge about groups of organisms despite the vital inputs, seductively authoritative-looking dendrograms, and current pre-eminence among taxonomy methodologies. The best role for phylogenetic techniques should be as partners with the much broader (and usually older) perspectives gained by a thorough understanding of the overall biology as the means to determine the most sensible and best supported organismal classifications.

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- Gryganskyi AP, Humber RA, Smith ME, Miadlikovska J., Wu S, Voigt K, Walther G, Anishchenko IM, Vilgalys R. 2012. Molecular phylogeny of the Entomophthoromycota. *Mol. Phylog. Evol.* 65: 682–694. <http://dx.doi.org/10.1016/j.ympev.2012.07.026>
- Gryganskyi AP, Humber RA, Smith ME, Hodge K, Huang B, Voigt K, Vilgalys R. 2012. Phylogenetic lineages in Entomophthoromycota. *Persoonia*: in press.

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***Moniliophthora aurantiaca* sp. nov.,
a Polynesian species occurring in littoral forests**BRADLEY R. KROPP^{1*} & STEVEN ALBEE-SCOTT²¹*Biology Department, 5305 Old Main Hill, Utah State University, Logan, Utah 84322 USA*²*Wilbur L. Dungy Endowed Chair of the Sciences, Environmental Sciences,**Jackson Community College, 2111 Emmons Road, Jackson, MI USA**CORRESPONDENCE TO: brad.kropp@usu.edu

ABSTRACT — A new species of *Moniliophthora* is described from the Samoan Islands. The new species is characterized by its bright orange pileus and pale orange stipe and lamellae. It occurs commonly on woody debris in moist littoral forests and has not been found in upland forests. A phylogenetic analysis of nLSU and ITS sequences indicates that *Moniliophthora aurantiaca* has an affinity with the Central and South American members of the genus. Possible mechanisms for the dispersal of fungi from the Neotropics to the Samoan Islands are discussed.

KEY WORDS — *Agaricales*, American Samoa, *Crinipellis*, Oceania, phylogeny

Introduction

The mycobiota of the Samoan Islands has received very little attention. What work that has been done consists of an inventory of the wood decay fungi and plant pathogens present in American Samoa (Brooks 2004, 2006) along with the recent description of an *Inocybe* species from the island of Ta'u (Kropp & Albee-Scott 2010). Our preliminary work on Samoan fungi indicates that the mycoflora of the islands is potentially quite rich and that a number of undescribed species is present.

An attractive, bright orange species of *Moniliophthora* was recently discovered in littoral forest on the islands of Tutuila and Ta'u, American Samoa. In spite of its distinctive appearance and the fact that it appeared to be very common at the time the material was collected, no name has been found for this fungus among the published species of the *Moniliophthora*/*Crinipellis* complex. In this article, we propose a new species of *Moniliophthora* from the Samoan Islands and assess its phylogenetic position relative to other members of *Moniliophthora* and to members of *Crinipellis* and *Chaetocalathus*.

Materials & methods

Specimens were photographed in the field on natural substrates or using a gray card as a background and field notes were taken while the material was still fresh. The collections were then dried for herbarium specimens. Microscopic study of the dried specimens was carried out in the laboratory using a light microscope after rehydrating tissue sections in Melzer's reagent. Microscopic characters were measured using oil immersion at 1000× except for pileus hairs, which because of their length were made at lower magnifications. All microscopical characters were illustrated with the aid of a drawing tube. Spore measurements are given as an average with ranges, whereas measurements of the other cells are given as ranges. Color notations were taken from the digital photographs using the Munsell Soil Color Charts (Munsell Color 2000) and Farver i Farver (Wanscher & Kornerup 1991). Formats for the Munsell and Farver i Farver color notations are 5YR 8/8 and 54A respectively. All specimens examined, including the holotype, have been accessioned into the Intermountain Herbarium (UTC) at Utah State University.

DNA was extracted from dried herbarium material using standard protocols adapted for use in our lab (Kropp et al. 1996). Amplified PCR products were obtained using standard PCR protocols (White et al. 1990) for the internal transcribed spacer (ITS) using primers ITS4 and ITS5 (White et al. 1990) and for the nuclear large ribosomal subunit (nLSU) between primers LROR and LR5 (Moncalvo et al. 2000). Direct sequencing of the purified PCR products was done for both the nLSU and the ITS using the amplification primers. Both sequences for *M. aurantiaca* were deposited in GenBank (ITS = JN692482, nLSU = JN692483).

Twenty-one species of *Crinipellis* representing Europe, Australasia, and North and South America were sampled. Taxon sampling also included four *Moniliophthora* species from Australasia, North America, and the American tropics. To identify apomorphies, members of the closely related *Chaetocalathus* and representatives of the less closely related *Tetrapyrgos* and *Marasmius* were used as outgroups in our analysis (TABLE I).

Clustal X (Thompson et al. 1997) was used to align the nLSU and ITS sequences after they were concatenated in the phylogenetically uninformative terminal ends. MrBayes 3.1 was used to analyze the aligned data set, 1613 bases in length (Ronquist & Huelsenbeck 2003). Treespace was searched using a time reversible model of evolution (Maddison 1994, Rodriguez et al. 1990) and a discrete gamma distribution with six substitution types and some invariant sites (GTR+G+I). Every hundred trees were sampled from the Bayesian simulation to approximate posterior probabilities. The trees were simulated using the Markov Chain Monte Carlo Method (MCMC) and all Bayesian simulations were conducted with eight active MCMC chains, heated at 0.2, and started with a randomly chosen neighbor-joining tree. The first MCMC run was iterated for 1,000,000 generations and three subsequent MCMC simulations were done using 1,000,000 generations, sampling every one hundredth tree. A majority consensus tree was calculated from the last 7000 sampled trees from a 10,000 tree data set using all runs to recover the posterior probabilities of the internal nodes using the `sumt` command in MrBayes. All four consensus trees recovered from the four Bayesian simulations were phylogenetically identical. The resulting statistics showed that the two independent MCMC chains converged with a standard deviation <0.001 (Ronquist & Huelsenbeck

TABLE 1. Species, geographic origins, and GenBank accession numbers

TAXON	ORIGIN	ITS	nLSU
<i>Chaetocalathus columellifer</i> (Berk.) Singer	Malaysia	FJ167665	FJ167665
<i>Ch. cf. columellifer</i>	Ecuador	AY916686	—
<i>Ch. craterellus</i> (Durieu & Lév.) Singer	Italy	FJ167664	—
<i>Ch. fragilis</i> (Pat.) Singer	Thailand	FJ167662	—
<i>Ch. galeatus</i> (Berk. & M.A. Curtis) Singer	Thailand	FJ167663	—
<i>Ch. liliputianus</i> (Mont.) Singer	Puerto Rico	AY916682	AY916680
<i>Ch. magnus</i> Halling	Colombia	FJ167666	—
<i>Crinipellis actinophora</i> (Berk. & Broome) Singer	Malaysia	FJ167626	—
<i>Cr. brasiliensis</i> Arruda et al.	Brazil	AY317137	—
<i>Cr. brunneipurpurea</i> Corner	Indonesia	FJ167646	—
<i>Cr. brunnescens</i> Kerekes & Desjardin	Indonesia	FJ167627	—
<i>Cr. campanella</i> (Peck) Singer	—	—	AF042647
<i>Cr. cupreostipes</i> Kerekes et al.	Thailand	FJ167641	—
<i>Cr. dipteroearpi</i> Singer	Thailand	FJ167651	—
<i>Cr. furcata</i> Kerekes et al.	Indonesia	FJ167658	—
<i>Cr. cf. iopus</i> Singer	Papua NG	FJ167639	—
<i>Cr. malesiana</i> Kerekes et al.	Malaysia	FJ167629	—
<i>Cr. maxima</i> A.H. Sm. & M.B. Walters	—	—	AF042630
<i>Cr. piceae</i> Singer	N. America	FJ167633	—
<i>Cr. procera</i> G. Stev.	New Zealand	FJ167660	—
<i>Cr. scabella</i> (Alb. & Schwein.) Murrill	Europe	FJ167635	AM946420*
<i>Cr. setipes</i> (Peck) Singer	Thailand/USA	FJ167634	AY916689**
<i>Cr. stipitaria</i> (Fr.) Pat.	Germany	AY571033	AY207194
<i>Crinipellis</i> sp.	Thailand	AY916698	—
<i>Crinipellis</i> sp.	Guyana	AY916701	—
<i>Cr. tabtim</i> Kerekes et al.	Thailand	FJ167643	—
<i>Cr. trichialis</i> (Lév.) Pat.	Indonesia	FJ167609	—
<i>Cr. zonata</i> (Peck) Sacc.	Canada	AY916692	AY916690
<i>Marasmius apatelius</i> Singer	Thailand	EU935561	—
<i>M. leucorotalis</i> Singer	Malaysia	FJ431253	—
<i>M. rotula</i> (Scop.) Fr.	N. America	DQ182506	DQ457686
<i>Moniliophthora aurantiaca</i>	Samoa	JN692482	JN692483
<i>M. canescens</i> (Har. Takah.) Kerekes & Desjardin	Malaysia	FJ167668	—
<i>M. pernicioso</i> (Stahel) Aime & Phillips-Mora	Peru	AF335590	—
<i>M. roreri</i> (Cif.) H. C. Evans et al.	Costa Rica	AY916746	AY916744
<i>Moniliophthora</i> sp.	N. America	AY916754	—
<i>Tetrapyrgos nigripes</i> (Fr.) Horak	N. America	DQ449942	AF261337
<i>T. subcinerea</i> (Berk. & Broome) Horak	S. E. Asia	EF175552	—
<i>T. subdendrophora</i> (Redhead) Horak	N. America	EF175521	—

* ITS sequence from a German collection, nLSU from an Estonian collection

**ITS sequence originated from a Thai collection, nLSU sequence from a North American collection.

2003). The potential scale reduction factors for all convergence statistics approached 1.001 for all parameters. Posterior probability support measures <50% are not shown. *Tetrapyrgos* was used to root the consensus tree.

Results

The results of the Bayesian analysis show that the undescribed species clusters with the *Moniliophthora* clade, which has very good posterior probability support (FIG. 1). Certain other taxa, such as *Crinipellis* aff. *iopus* and *Crinipellis brasiliensis* (which have not yet been transferred to *Moniliophthora*), also clustered within this clade. Our analysis indicates that *M. aurantiaca* has a stronger affinity with the neotropical members of the clade such as *M. roreri* than it does with the Australasian representatives such as *M. canescens* or *Crinipellis* aff. *iopus*. *Moniliophthora aurantiaca* is closely related to *M. roreri*, which has a neotropical distribution. In addition, two other neotropical taxa, *M. perniciosa* and *Crinipellis brasiliensis*, appear to be close relatives of *M. aurantiaca* based on our analyses.

The species of *Chaetocalathus* and *Marasmius* formed two well-supported clades basal to *Crinipellis* and *Moniliophthora*. In our analysis, the members of *Moniliophthora* occupy a clade derived from within *Crinipellis*. Based on these results, accepting *Moniliophthora* as a genus renders *Crinipellis* paraphyletic, although the node separating *Crinipellis* clade 1 from *Crinipellis* clade 2 and *Moniliophthora* is not well supported (FIG. 1).

Discussion

Moniliophthora aurantiaca is an unusually conspicuous element of the mycobiota of American Samoa. It stands out primarily because of its bright orange colors but also because it fruits commonly in littoral forests. Thus far, it has been found only in a narrow strip of damp littoral forest situated along the shoreline on the islands of Tutuila and Ta'u, American Samoa. In one instance, it was found among trees growing on a rocky outcrop a few meters above the water but still at the shoreline. That *M. aurantiaca* was never found outside the littoral zone in spite of an abundance of apparently suitable moist woody substrates in nearby higher elevation forests suggests a halophilic or maritime beach ecology. Known thus far only from American Samoa, *M. aurantiaca* may be endemic to the Samoan Islands, but its distribution is still poorly known and further work is needed to confirm whether it is really an endemic species.

The species belongs to the *Moniliophthora*/*Crinipellis* complex that currently comprises five *Moniliophthora* species and 163 *Crinipellis* species (<http://www.indexfungorum.org/>). Of these, *M. aurantiaca* macroscopically most closely resembles the South American species *Crinipellis ticoi* Halling, which is also orange in color. Despite their macroscopic similarities, *C. ticoi* differs from *M. aurantiaca* microscopically in larger spores ($12.1\text{--}14.3 \times 5\text{--}7.1 \mu\text{m}$) and

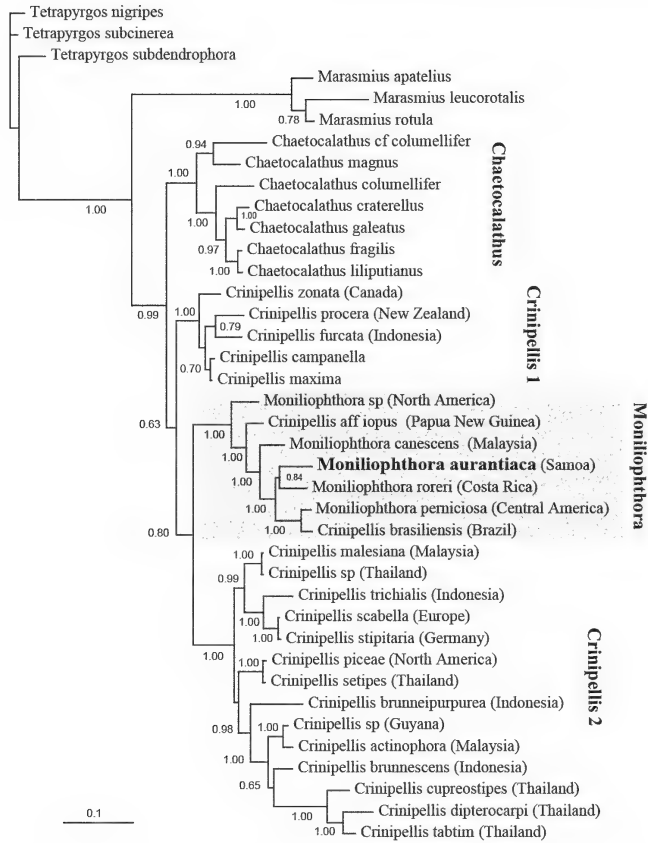


FIGURE 1. Phylogram from a Bayesian analysis of a combined ITS and nLSU dataset for *Crinipellis*, *Moniliophthora*, and *Chaetocalathus*. Support measures are shown for nodes with 60 percent or greater posterior probability support. Members of *Tetrapyrgos* were used to root the phylogram.

cheilocystidia that lack the apical appendages present on the cheilocystidia of *M. aurantiaca* (FIG. 3). One other orange *Crinipellis* is the African *C. hygrocyboides* (Henn.) Singer. Neither Hennings (1902) nor Singer (1989) gives microscopic details or a detailed description for this fungus, making comparisons with other taxa difficult. Since Halling (1993) reports that spores and cystidia were not observed in the type material of *C. hygrocyboides* from the Muséum National d'Histoire Naturelle, we have studied isotype material of *C. hygrocyboides* (= *Marasmius hygrocyboides* Henn.) from the Royal Botanical Gardens, Kew. The specimen resembles *M. aurantiaca* but differs by having a mixture of long dextrinoid hairs and clavate, thin-walled dextrinoid cystidia on the stipe surface and similar, but slightly smaller, spores. We were unable to

reliably study cheilocystidia in the *C. hygrocyboides* material due to difficulty reviving the material. However, all cells at the lamellar edges appeared to be clavate and different from the cheilocystidia with apical appendages of *M. aurantiaca*. It would be very useful to have molecular data for this species to determine whether it belongs to the *Moniliophthora* clade. Another similar taxon is *M. canescens*, which has orange hues when wet but is typically brownish; additional differences include an initially white stipe that becomes brown and narrower spores (Takahashi 2000). *Crinipellis malesiana*, which also develops orange colors in age (Kerekes & Desjardin 2009), is phylogenetically separate from *M. aurantiaca* (FIG. 1).

Based on our phylogenetic analysis, *M. aurantiaca* falls within the *Moniliophthora* clade of Aime & Phillips-Mora (2005). These workers found that fungi belonging to *Moniliophthora*, which had been used for an anamorphic pathogen of cocoa (Evans et al. 1978), are not only closely allied to certain members of *Crinipellis* but form a distinct lineage with *Crinipellis* in the *Marasmiaceae* for which they proposed using *Moniliophthora* as a generic name. The genus currently includes both conidial and agaricoid taxa. The agaricoid members of *Moniliophthora* are usually relatively brightly colored species that would otherwise have been placed in section *Iopodinae* of *Crinipellis*.

In contrast, our analysis indicates that *Moniliophthora* does not occupy a distinct lineage but is derived from within *Crinipellis*. In this case, accepting *Moniliophthora* as a genus makes *Crinipellis* paraphyletic (FIG. 1). Based on our results, one could abandon *Moniliophthora* and transfer everything in it, including the anamorphic species, to *Crinipellis*. However, one cannot ignore that the ITS analysis of Kerekes & Desjardin (2009) and the 5-gene phylogeny of Aime & Phillips-Mora (2005) both agree that the *Moniliophthora* clade forms a distinct lineage. In addition, the node separating the two *Crinipellis* clades shown in our phylogram has weak posterior probability support (FIG. 1). Given the discrepancy between our analysis and the other two studies, further work is needed to clarify relationships in the *Moniliophthora*/*Crinipellis* complex. For now, the preponderant information indicates that the *Moniliophthora* clade forms a distinct lineage and until further work is done we retain *Moniliophthora* as a genus in the sense of Aime & Phillips-Mora (2005).

Caution must be used when using our phylogram to identify biogeographical patterns. Nonetheless, the close affinity of *M. aurantiaca* to the pathogenic South and Central American members of the *Moniliophthora* clade is well supported and implies that either *M. aurantiaca* or an ancestral taxon originated in the neotropics (FIG. 1). This is somewhat unexpected, because we earlier found that another Samoan fungus, *Inocybe tauensis* Kropp & Albee-Scott (2010), has paleotropical roots and because Samoan plant communities have Australasian ties (Van Balgooy et al. 1996).

Regardless of where *M. aurantiaca* originated, long-distance dispersal was clearly involved because the islands are relatively young and have never been connected to a land mass (McDougall 1985). In recent times, humans could have carried *M. aurantiaca* to the Samoan Islands on plants or in woody materials from almost any part of the world. However, long-distance dispersal might also have occurred via other well-known mechanisms. Rafting is one potential means of transport because *M. aurantiaca* grows in woody debris and could be carried long distances by floating wood. Rafting is known to transport organisms and is thought to have resulted in the dispersal and subsequent cladogenesis of snails across large areas of the Pacific Ocean, including an endemic Samoan snail (Donald et al. 2005). In another instance, an oyster species is thought to have been carried via rafting from Chile to New Zealand (Foighil et al. 1999). Thus, a plausible mechanism exists for the dispersal of fungi from the Neotropics to Samoa. The mycelium of *M. aurantiaca* could have been carried within woody material from South America to Samoa via the South Equatorial Current that has a general westward flow across tropical portions of the South Pacific. Air-borne spores are another means by which agarics could potentially cross the Pacific and it is clear that fungal spores can travel long distances with air masses (Brown & Hovmöller 2002). If *M. aurantiaca* has neotropical roots, then its spores or those of an ancestral taxon would have needed to cross the Pacific from the east to get to the Samoan Islands. The easterly trade winds of the equatorial South Pacific provide another plausible mechanism by which this could have happened.

Taxonomy

Moniliophthora aurantiaca Kropp & Albee-Scott, sp. nov.

FIGS 2, 3

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Differs from *Crinipellis ticoi* by its smaller basidiospores and cheilocystidia with apical appendages and from *Crinipellis hygrocyboides* by its short, moderately thick-walled setae-like stipe hairs and the absence of clavate caulocystidia.

TYPE: American Samoa, National Park of American Samoa, Ta'u Unit, in littoral forest along trail to Si'u point, on woody debris, 17 May 2009, leg. B.R. Kropp BK17-May-2009-20a (Holotype UTC253631; GenBank JN692482, JN692483).

ETYMOLOGY: *aurantiaca* refers to the bright orange coloration of the pileus.

PILEUS 3–15 mm diameter, convex with margin somewhat inrolled at first then broadly convex to nearly plane, disc with a small umbo, sometimes slightly depressed around the umbo, translucent-striate or slightly sulcate from the margin nearly to the disk, surface matted fibrillose; bright red orange (78A, 2.5YR 7/12) at the center, becoming pale orange (54A, 5YR 8/8) toward the margin especially in age; when dry, pale orange with the umbo darker. LAMELLAE narrowly attached, subdistant, pale orange (65A, 5YR 8/8), very

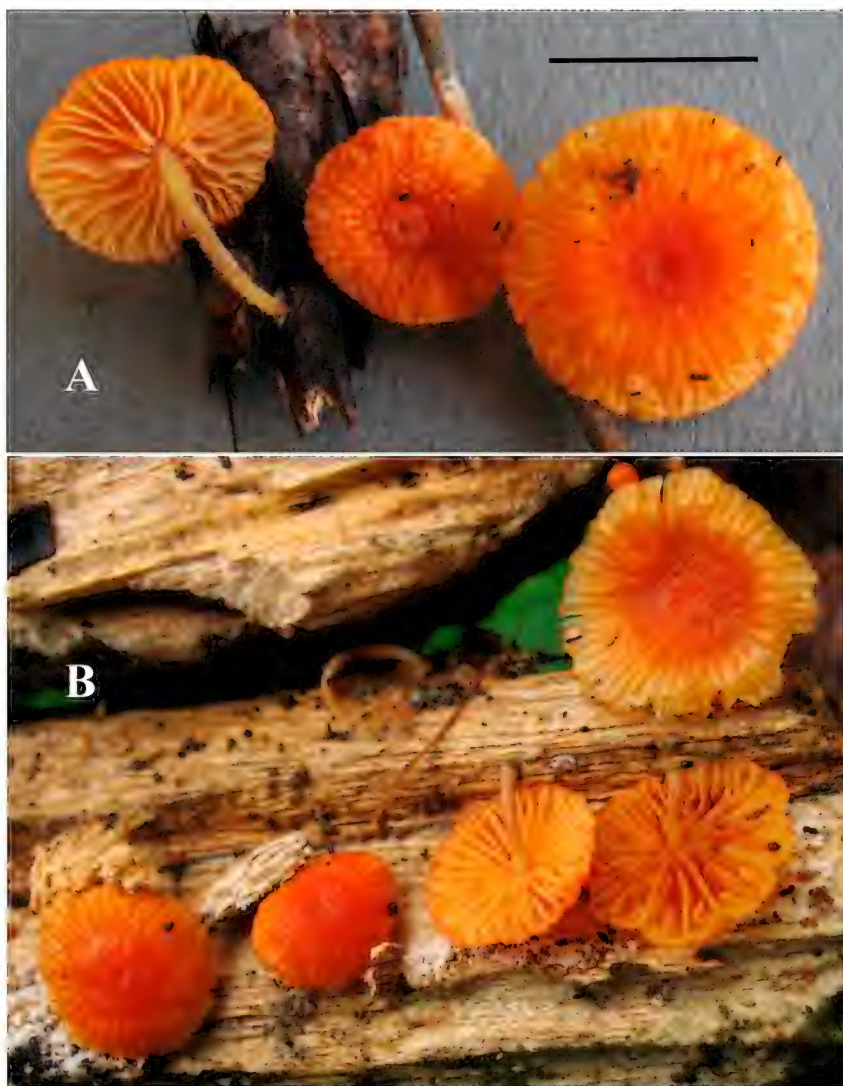


FIGURE 2. *Moniliophthora aurantiaca*. Basidiomata: A. UTC253824; B. UTC253809. Scale = 1 cm.

pale orange when dry; lamellulae in 1–2 series. STIPE 5–8 × 0.25–1 mm, central, terete, equal, densely pruinose, concolorous with the lamellae except for the base which is often brown, extreme base on some specimens strigose, stipe inserted for most specimens. PILEIPELLIS a cutis of fairly loosely woven light brown, lightly encrusted hyphae 5–8 μ m wide, not well differentiated

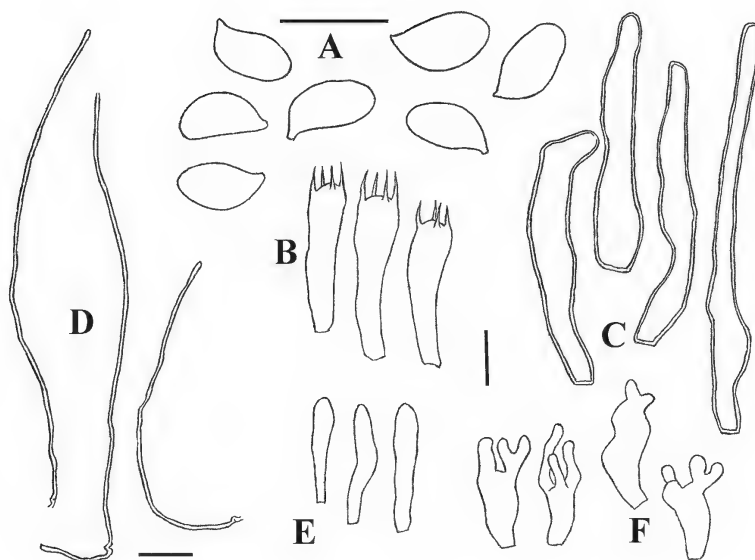


FIGURE 3. *Moniliophthora aurantiaca*. A. Basidiospores B. Basidia C. Stipe hairs D. Pileipellis hairs E. Basidioles F. Cheilocystidia. Scales: D = 50 μ m; A–C, E, F = 10 μ m.

from the pileus context; numerous long, dextrinoid hairs present, these with a basal clamp and $400\text{--}600 \times 4\text{--}5 \mu\text{m}$, mostly hyaline not greenish in KOH; context not dextrinoid. LAMELLAR TRAMA subparallel to interwoven; hyphae hyaline sometimes lightly encrusted, clamped, $4\text{--}8 \mu\text{m}$ wide; context not dextrinoid. STIPE TISSUE parallel, smooth, hyaline, hyphae $4\text{--}8 \mu\text{m}$ wide; dextrinoid to weakly dextrinoid; stipe hairs numerous, covering the stipe apex and base, dextrinoid to weakly dextrinoid, short and moderately thick-walled, resembling setae, $52\text{--}85 \times 5\text{--}10 \mu\text{m}$. BASIDIOSPORES $7.5\text{--}(8.7)\text{--}11.0 \times 4\text{--}(4.6)\text{--}6.0 \mu\text{m}$, $Q = 1.8\text{--}2.1$, amygdaliform, thin-walled, smooth, hyaline, inamyloid. BASIDIA $26\text{--}30 \times 6\text{--}7 \mu\text{m}$, clavate, typically four-spored. BASIDIOLES $22\text{--}25 \times 4\text{--}5 \mu\text{m}$, clavate. PLEUROCYSTIDIA none. CHEILOCYSTIDIA $20\text{--}22 \times 5\text{--}8 \mu\text{m}$, numerous, hyaline, thin-walled, typically with several irregular apical, finger-like appendages. CLAMPS present. RHIZOMORPHS absent.

HABITAT AND DISTRIBUTION occurring on fallen twigs and other woody material in littoral forest.

ADDITIONAL SPECIMENS EXAMINED—AMERICAN SAMOA. TUTUILA, near Vaitogi, on woody debris in wooded area near shoreline, 11 May 2009, leg. B.R. Kropp, BK11-May-2009-11 (UTC253809); National Park of American Samoa, Tutuila Unit, on woody debris in littoral forest along trail to Pola Island, 12 May 2009, leg. B.R. Kropp, BK12-

May-2009-12 (UTC253824); BK12-May-2009-16 (UTC253828); BK12-May-2009-18 (UTC253830); BK12-May-2009-17 (UTC253829). TA'U, National Park of American Samoa, Ta'u Unit, on woody debris in littoral forest along trail to Si'u point, 15 May 2009, leg. B.R. Kropp, BK15-May-2009-10a (UTC255959).

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MYCOTAXON

<http://dx.doi.org/10.5248/119.505>

Volume 120, pp. 505–506

April–June 2012

Regional annotated mycobiotas new to the Mycotaxon website

ABSTRACT — Five new species distribution lists are added to the MYCOTAXON ‘web-list’ page covering *Coprinaceae* & *Strophariaceae* and *Bolbitiaceae* & *Crepidotaceae* in Argentina (both by Niveiro & Albertó); macromycetes from Collestrada forest ecosystems in Perugia, Italy (by Angelina & al.); lichens from Pollino National Park, Italy (by Puntillo & al.); and saprobes on *Alnus alnobetula* in the Swiss alps (by Senn-Irlet & al.). This brings to 94 the free access mycobiotas now available on the MYCOTAXON website <<http://www.mycotaxon.com/resources/weblists.html>>.

SOUTH AMERICA

Argentina

Niveiro, N.; Albertó, E. Checklist of the Argentinean *Agaricales* 2. *Coprinaceae* and *Strophariaceae*. 38 p.

ABSTRACT — A checklist of species belonging to the families *Coprinaceae* and *Strophariaceae* was made for Argentina. The list includes all species published till year 2011. Twenty-one genera and 251 species were recorded, 121 species from the family *Coprinaceae* and 130 from *Strophariaceae*.

Niveiro N. & E. Albertó. Checklist of the Argentine *Agaricales* 3. *Bolbitiaceae* and *Crepidotaceae*. 28 p.

ABSTRACT — A checklist of *Agaricales* species belonging to *Bolbitiaceae* and *Crepidotaceae* has been made for Argentina. The list included all species published until 2011. Fourteen genera and 187 species are recorded, 94 species from the *Bolbitiaceae* and 93 from *Crepidotaceae*.

EUROPE

Italy

Angelini, Paola, Giancarlo Bistocchi, Andrea Arcangeli & Roberto Venanzoni. Preliminary checklist of the macromycetes from Collestrada forest ecosystems in Perugia (Italy). 14 p.

ABSTRACT — A preliminary taxonomic list of the macromycetes growing in forest ecosystems in Perugia (Italy) is presented based on mycological research carried

out in the most widespread local plant communities from the forest of Collestrada: *Quercus* spp. woodlands, *Carpinus betulus* L. woodland and plantations with *Pinus pinea* e/o *Pinus pinaster*. In the period from Jan. 2011 to Dec. 2011 133 taxa belonging to 70 genera were recorded. For each taxa the following items were reported: Latin name, author, WGS-84 Global Position System (GPS) coordinates, coordinate grids from a Google Earth Collestrada image, date of the survey and habitat. This work contributes to the Umbrian regional checklist, which will eventually be integrated with the Italian national checklist.

Puntillo, D., M. Puntillo, G. Potenza & S. Fascetti. New, rare, and noteworthy lichens in the Pollino National Park (Basilicata, southern Italy). 13 pp.

ABSTRACT — 42 lichens new to the Basilicata Region were found in the territory of the Pollino National Park (southern Italy). There are two very interesting groups of species: the first, the *Caliciales*, indicator of the ecological continuity of the forest, are present on old trees (*Abies alba* Mill.). The second one consists of foliicolous species that are present in a small humid valley with subtropical microclimatic conditions. From the results of this work, the number of *taxa* of the lichen flora of Basilicata rises to 558 species, with an increase of 9%.

Switzerland

Senn-Irlet, Beatrice, Rolf Mürner, Elia Martini, Nicolas Küffer, Romano de Marchi & Guido Bieri. Saprobic fungi on wood and litter of *Alnus alnobetula* in the Swiss Alps. 34 pp.

ABSTRACT — 246 species representing 73 genera and 90 species of ascomycetes, basidiomycetes being represented with 44 genera of aphyllophoralean fungi with 77 species, 23 genera of agarics with 68 species and 8 genera of tremelloid fungi with 12 species growing on wood and litter of *Alnus alnobetula* in Switzerland are given. *Clitocybe* and *Mycena* species dominate among the leaf litter inhabiting species. Fallen branches have the highest species diversity. The host-specific *Peniophora aurantiaca* is one of the most conspicuous and most frequent species.

MYCOTAXON

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